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AN AMPHILINID CESTODE FROM AN AUSTRALIAN TORTOISE

by

T. HARVEY JOHNSTON

(University of Adelaide).

(Submitted for publication 7th November, 1930.)

Three cestodes, collected early in 1930 by Mr. W. Filmer, from a fresh-water tortoise, *Chelodina longicollis,* at Tuggerah, New South Wales, were submitted recently by the South Australian Museum for identification. Mr. Filmer, in reply to a series of questions, stated that, when removing the viscera from a female tortoise, he found the worms, which resembled eggs in shape. They were yellow when alive, with a markedly tessellated appearance, rounded in form, but slowly elongating each extremity until the worms became fully extended and flat. They were placed in 50% alcohol for preservation. The normal habitat is probably the coelome. In view of the special interest attaching to Cestodaria, of which so few species are known, Mr. Filmer's find is a very fortunate one, and a preliminary account of the parasite is now offered. This seems to be the first occasion on which an Amphilinid has been taken in the southern hemisphere, and is the only known occurrence of a member of the order in a reptile, the few remaining species having been described, one from sturgeons in the northern hemisphere, two from a primitive fresh-water fish from northern South America, and one from a marine fish off Ceylon.

The worms measure 60 by 10 mm., 68 by 7·5 mm., and 68 by 10 mm. in maximum dimensions. The anterior end is rather pointed, terminating in a short, partly retracted rostellum. The body widens rather rapidly for the first quarter or third of the length and the breadth then remains more or less constant almost to the opposite end. In the vicinity of the midregion of the receptaculum seminis, the body narrows to terminate in a rounded extremity bearing a small depression, which receives the sex ducts. The parasites are thin, the greatest thickness occurring in the vicinity of the receptaculum. The surface is markedly tessellated, or rather pitted, there being abundant diamond-shaped concavities separated by wavy ridges. This net-like arrangement is somewhat like that described for *Amphilina foliacea*, and rather more regular than that of *Nesolecithus* as figured by Janicki (3) under the name of *Amphilina liguloidea.*

The rostellum is essentially like that of the species just named, except that it is much larger in its introverted condition, and measures about a millimetre in length and 0·25 mm. across its base, where the retractor muscles are inserted. The uterine pore opens near the base of the rostellar cavity.

Below the delicate cuticle and subcuticular region are abundant fine longitudinal muscle fibres, which tend to become aggregated here and there into small bundles. These are succeeded by diagonal fibres and by well-marked transverse fibres, which are closely arranged anteriorly. Dorso-ventral fibres are very abundant, but are arranged in small clusters. A thick layer of fine fibres accompanies the uterus, conforming to its changes in size and shape, while similar, though fewer, fibres surround the ovary and receptaculum.

The excretory and nervous systems have not been traced, though numerous canals can be seen, indicating that the former system probably resembles that of *Amphilina* as described by Hein (2). Large finely granular cells are scattered in the parenchyma in the anterior part of the worm, and no doubt represent the frontal glands of other Amphilinids. They lie mainly in front of the level of the vitelline glands. Calcareous corpuscles are distributed here and there in the parenchyma.

The orientation of the parasite has been a matter of difficulty, since the sex apertures are all terminal and the testes are distributed in the upper and lower portions of the parenchyma, while the ovary lies more or less in the middle of the medullary region. The ovary is assumed to be on the right of the midline, as is indicated in Monticelli's (4) figure of *Amphilina liguloidea* (Dies.).

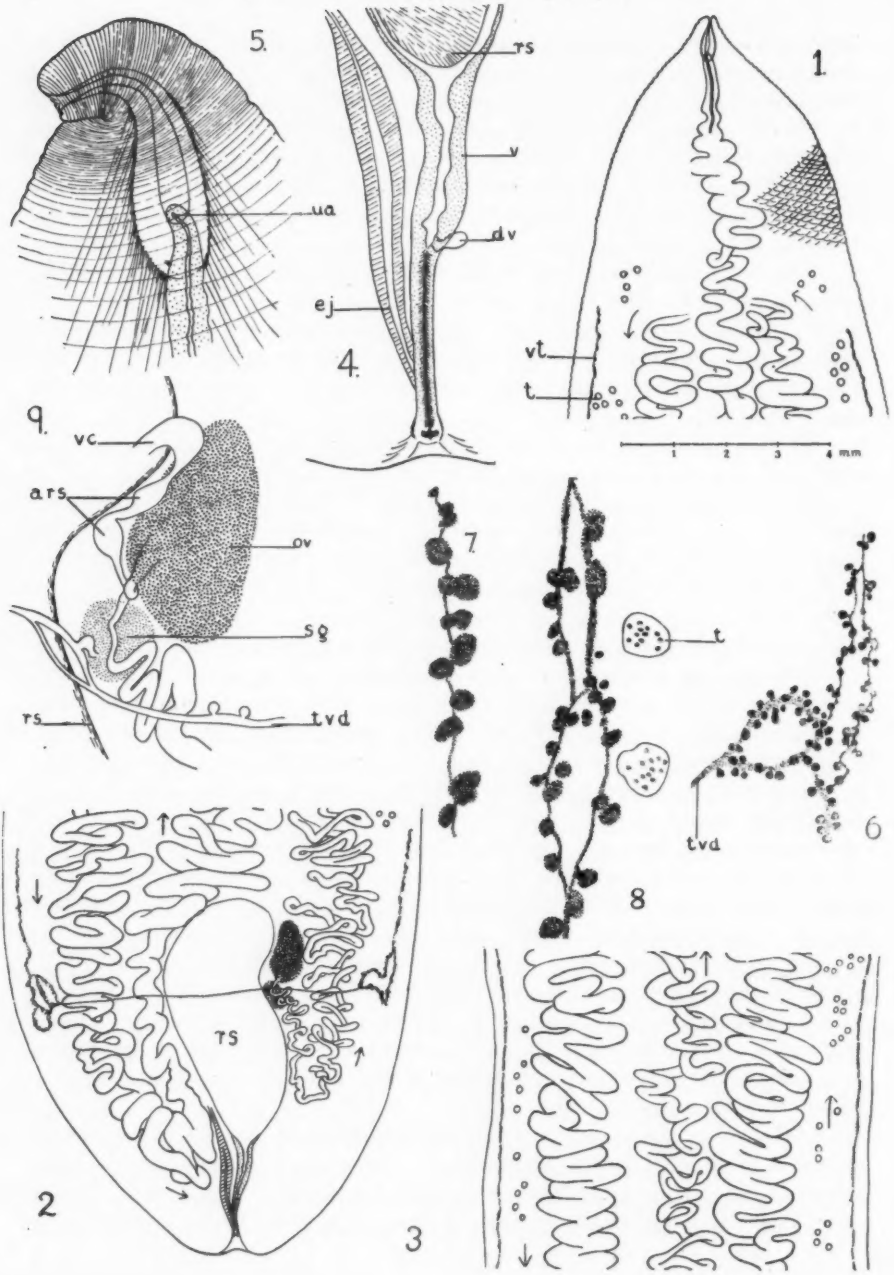
The testes are numerous, especially laterally between the vitellaria and the uterus, where they form two layers. They occur above and below the three stems of the uterus, but are less abundant in these positions. They extend posteriorly almost to the level of the anterior end of the receptaculum seminis and the posterior limit of the vitelline vesicles, while anteriorly they occur plentifully in the region in front of the vitellaria, as far forward as the point where the first ascending stem of the uterus bends across to the other side of the parasite. They appear as hollow organs, about 0·15 to 0·2 mm. in length by 0·12 to 0·2 mm. in width. In one case a narrow tube containing sperms was seen to pass inwards from a vesicle, but the further course of this vas efferens was not traceable. Here and there in the lateral fields portions of a curved vas deferens are recognizable, but the general relationships of the duct were not observed. There is no obvious vesicula seminalis such as that described in some other Amphilinids. The two vasa probably unite as delicate ducts in the vicinity of the middle of the receptaculum to form a tube which travels posteriorly very close to the receptaculum on the side opposite to the ovary. This becomes slightly spindle-shaped, and its walls are greatly thickened and well provided with muscular tissue as well as prostate cells. This ejacu-

latory organ, the "propulsionsapparat" of Janicki, travels backwardly in an almost straight course close to the vagina and between the latter and the rearmost coils of the uterus. The two sex ducts converge towards their extremities, the female coming to lie above the other. The apertures open beside each other into a definite depression at the end of the parasite, provided with sphincter fibres. This species is the only known Amphilinid in which the two openings terminate together at the posterior end of the worm. A cirrus sac was not recognized.

The ovary lies immediately to the right of the inner end of the receptaculum as a rather flat structure of rounded outline, with its longer axis approximately parallel with that of the receptaculum. In the very narrow region between these two organs lie the vaginal canal and its enlargements, and also the shell gland complex, the latter being situated on the left of the posterior end of the ovary.

From a hollow on the inner posterior face of the latter organ the short oviduct arises and passes backwards and inwards, becoming markedly curved to join the vaginal canal just before entering the flat shell gland. Within the latter the ootyp is thrown into several coils (not indicated in the figure), and finally emerges ventrally as a very narrow uterine duct, which, in the vicinity of the transverse yolk duct, becomes widened and then thrown into a series of open coils or loops, occupying a considerable area to the right of the mid-region of the receptaculum, behind the level of the posterior end of the ovary. The tube then becomes bent forward as the first ascending limb, which is thrown into rather long loops lying mainly transversely and close together, somewhat as in *Gigantolina magna* (Southwell). The inner ends of some of these loops almost touch those of the median limb. Towards its anterior limit the loops become shorter and approximate the midline, and then the tube crosses over to the opposite side at about 5 millimetres from the anterior end. It possesses a similar course, but now as a descending limb, which eventually reaches nearly to the posterior end, extending a short distance beyond the receptaculum and a considerable distance behind the most posterior loops of the first limb. It then ascends in a series of loops or coils close to the receptaculum, assuming a median position in front of the latter to form the median ascending limb, whose folds are shorter and wider than those of the other two, and eventually terminate in a very thick-walled, wavy tube, or metraterm, with a narrow lumen, and containing a single row of eggs. This opens at the side of the base of the introverted rostellum. Some of the uterine loops may overlie the ejaculatory duct and underlie a part of the receptaculum.

The position of descending and second ascending stems is distinct from that described in all other known Amphilinids. The descending limb is on the right side of the midline in *Amphilina;* on the left of it in *Nesolecithus,* where the first ascending stem is practically median; on the left in *Schizochoerus;* and median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in *Gigantolina*. The second ascending stem is on the left in all these four genera. The uterus opens on the left side of the terminal rostellum in *Amphilina*. according to the figures of Hein (2) and Cohn (1), though Wagener (8) indicated the pore as being subterminal and lying on the right of the "sucker." According to Monticelli (4) the uterine aperture is on the right side of the "anterior sucker" in *Schizochoerus*. Janicki (3) records it as being terminal along with the rostellar opening in *Schizochoerus* and *Nesolecithus*, so that it resembles the condition occurring in the Australian species. In *Gigantolina*, Southwell (7) states that the uterus terminates in a minute pore "at the base of the small anterior end of the worm," though his figure indicates it as being on the left side at a short distance from that extermity.

The vitelline system seems to be different from that described as occurring in other Amphilinids. It occupies a very narrow linear zone, at 0·2 to 0·3 mm. inwardly from each margin of the parasite. Each vitellarium consists of a large number of small follicles, more or less rounded and varying in size, arranged irregularly in a sessile fashion around a central duct. The organs extend almost as far forwards as the point of crossing of the uterine limbs, and posteriorly to the level of the shell glands. Here and there along its course the duct may split, the two parts joining again, somewhat as described for *Nesolecithus* by Janicki. This division of the ducts becomes more obvious at the posterior end of each gland, where the reunion gives rise to the transverse yolk duct which passes inwards below the uterine loops to meet its fellow on the right side in a double junction just behind the ovary and shell gland. The left transverse duct passes under the receptaculum also. The very narrow common duct curves forwards a short distance to enter the ootyp above the shell gland. A sheath of longitudinal muscle fibres more or less surrounds the longitudinal ducts.

The vagina travels inwards from the terminal pore in the vicinity of the median axis of the worm for about 2 mm. to enter the large receptaculum seminis. It is the only known Amphilinid possessing a median terminal vaginal aperture. The duct is narrower in its outer half, where its lumen is lined by abundant, relatively long, hairs set obliquely, with the free ends directed anteriorly. At the inner end of this portion there is a short diverticulum, somewhat coiled or curved, passing upwards, but a definite aperture was not recognized. This portion probably represents the dorsal branch of the vagina, such as occurs in Schizochoeridae. The inner part of the duct has thicker walls, a wider lumen, and a slightly curved course. The walls become thin and the lumen very greatly

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Fig. 1, Anterior end of worm; **2,** posterior end; **3,** midregion, all drawn to the same magnification (see scale below fig. 1); **4,** sex ducts at posterior end; **5,** rostellum; **6,** posterior portion of vitelline gland; **7,** usual condition of vitellarium; **8,** "island" formation of vitellarium; **9,** ovary, shell gland, and associated structures.

ars, accessory receptacula; d v, dorsal branch of vagina; e j, ejaculatory apparatus; ov, ovary; r s, receptaculum seminis; s g, shell gland; t, testis; t v d, transverse vitelline duct; u, uterus; u a, uterine aperture; v, vagina; v c, vaginal canal; v t, vitellarium.

widened as the receptaculum is entered. The latter is slightly bent towards either end in opposite directions, and measures about 5 mm. long by 1·8 mm. wide. In all specimens it is filled with a gelatinous material, probably coagulated prostate secretion and sperms. From the right side at its inner end there arises a duct, the vaginal canal, which curves backwards to lie in the narrow region between the ovary and the receptaculum, and in this locality it may form two small accessory receptacula, separated by narrow portions of the duct. From the second and more posterior of these two thin-walled, poorly staining structures a very short, narrow tube joins the oviduct in the vicinity of the shell gland. The vaginal canal lies rather ventrally to the level of the ovary.

Eggs are extremely abundant. The uterus may contain many ruptured shells and sometimes groups of embryonal hooklets, indicating the death of many embryos in utero. Eggs are oval, measuring 0·13 to 0·18 in length by 0·08 to 0·10 mm. in width. The egg shell is thin, and closely invests the lycophora. The latter resembles those of *Amphilina* and *Nesolecithus* as described by Salensky (6) and Janicki respectively, the longer set of gland cells staining readily with haemotoxylin, while the remainder do not. The ten hooklets are very slender, measure about 0·03 mm. long, and possess long, delicate roots.

In 1921 Poche (5) reviewed the known Amphilinids, added three new genera to the one previously known, and arranged the group in two families, assigning *Amphilina* to the Amphilinidae and the remainder to the Schizochoeridae, which he subdivided into two new subfamilies, Schizocherinae and Gigantolininae. To the former he allotted two genera, each containing one species, *Schizochoerus liguloidea* (Dies) and *Nesolecithus janickii* Poche (syn. *Amphilina liguloidea* Janicki nec Diesing). To the latter subfamily was assigned the single species, *Gigantolina magna* (Southwell), of which a corrected account was promised. As such, if published, is not available to the writer, Southwell's account has been used in comparisons. *Amphilina* occurs only in sturgeons, *Schizochoerus* and *Nesolecithus* only in the Osteoglossid fish, *Arapaima gigas*, while *Gigantolina magna* is the only species known to occur in a marine teleost, its host being *Diagramma crassispinum*.

The Australian species, for which the name *Austramphilina elongata* n.g., n.sp., is proposed, seems to lie nearer the Schizochoeridae than the Amphilinidae, but its characters are so distinctive that a new family, Austramphilinidae, is proposed for its reception.

The following diagnosis is suggested for the family: Amphilinidea with frontal glands restricted to the anterior end; very numerous testes, extending from one vitelline region to the other, and occurring above and below the three uterine stems; a rather long ejaculatory apparatus, apparently devoid of a penis; male aperture terminal; uterus with the descending stem on the opposite side to the first ascending stem, and with the second ascending stem median; uterine pore opening anteriorly into the cavity of the rostellum; receptaculum seminis

lying mainly behind the level of the ovary and devoid of a long median anterior extension; vagina with short dorsal diverticulum and opening medianly at the posterior end beside the male aperture. The characters, as set out, allow a ready differentiation of the new family from the Amphilinidae and Schizochoeridae, as diagnosed by Poche.

The generic characters may be set down thus: Austramphilinidae; with tesselated or net-like appearance, owing to the arrangement of superficial ridges; and with vitellaria composed each of a central duct, around which small sessile follicles are arranged. Type *A. elongata* n.sp., from the coelome of a freshwater tortoise, *Chelodina longicollis*, from New South Wales. The type slide is registered as W2784 in the collection of the Australian Museum, Sydney.

Acknowledgment is made to the Trustees of that Museum for the opportunity to study the species, and to Mr. W. Filmer for his kindness in supplying information.

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A DISEASE OF HOPS IN TASMANIA AND AN ACCOUNT OF A PROTOMYXEAN ORGANISM, LEPTOMYXA RETICULATA GOODEY var. HUMULI (nov. var.), ASSOCIATED WITH IT

by

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(Submitted for publication 24th October, 1930.)

Hop-growers in Tasmania have reported the occurrence of a disease in their hop gardens for the last ten years or more, and in 1927 the Commonwealth Council for Scientific and Industrial Research asked me to visit the hop areas in that island, with a view to undertaking an examination of the affected plants.

The garden inspected by me belonged to Mr. Rupert Shoobridge, and was situated at Glenora, in the Derwent Valley; it was typical of those in this district. The variety *Fuggles* is largely grown in the valley, and it seems to be very susceptible to a disease which the growers call "take-all." It is an English variety, and is an early hop—the first to be picked. An American variety, "Golden Cluster," is also grown in this garden and elsewhere; it appears to be more resistant to the trouble, but not entirely so. However, as this is a later hop, and in addition tends to decline in vigour rather quickly, it is not regarded with such favour by growers in Tasmania.

SYMPTOMS OF THE TASMANIAN DISEASE.

The disease manifests itself in the production of much weaker bines than those produced by normal hops; they rarely grow more than 6–9 feet in height, but occasionally definite disease symptoms may be delayed until they attain a height of 14–18 feet. The internodes do not lengthen as they should; the bines therefore do not reach the cross-wires and produce practically no hop cones, *but they do not fall away from their support.* The leaves of those bines which, although practically sterile, persist through the growing season tend to have their margins slightly incurved, and are of a rather darker green than the normal leaf, and on the average they are smaller in size. The majority of the leaves at some time tend to wither and shrivel. This is first noticeable at their margins, but the withering and browning gradually progress inwards, and are very typical of

the trouble. At a later date the leaves become entirely brown and dry, but they are not cast off from the plant. If mature when attacked the "hill" may not be absolutely killed for some years, but each year the growth tends to become more and more dwarfed and completely sterile. The leaves of these very dwarf bines remain small and rather crumpled, and incline towards a yellowish-green colour.

The root-system of infected plants, although the older spreading laterals were usually in a state of decay, showed no deformation comparable with the nodular swellings sometimes encountered in these hop gardens, due to the crown-gall organism. The two conditions are quite distinct. The diseased plants occur in patches through the garden. and these are not confined to any particular contour or aspect of it. Young or old hills seem to be equally susceptible to attack, but young hills succumb to the disease more quickly.

HISTORY OF NETTLEHEAD DISEASE OF HOPS.

These symptoms parallel rather closely those given by Duffield (12) when describing the appearance of hops suffering from "false nettlehead." He says: "The bine in this case usually attains the height of 5 feet in older hills, but in young hills it ceases to grow, and usually dies when only a foot or so high. As in the case of 'true nettlehead,' the leaves when fresh are in a crumpled state, but are considerably larger and of a bluish colour; the internodes also are foreshortened, giving the plant a bunched-up appearance. In this case the bine does *not* fall away from its support [in contrast to true nettlehead (mosaic)[1]], but after reaching a certain height the plant may either wilt and *die away* or remain fresh in a dormant state. . . . . The wilting of the bine has been noticed at the end of May [English conditions], but in the majority of cases it begins at the end of June, and by August *the foliage is quite dead and dry.*"

Duffield also notes that when the bine dies away in August *the root-system is in a state of decay*, in contrast to the condition found in true nettlehead (mosaic), where the bine remains green, though growth is retarded, and at the same time the root-system remains in an apparently healthy state. In comparing the two forms of nettlehead he also draws attention to the fact that the true form (mosaic) is usually to be found starting from the edge of a garden, especially where the garden slopes down to a deep ditch or high hedge. False nettlehead, on the other hand, he says, may be found distributed in patches throughout the garden.

This "nettly condition," without discrimination between true and false, was ascribed by Percival (28) to eel-worm attack *(Heterodera schachtii)*. Duffield, clearly recognizing the distinctions between the two forms of nettlehead, found no correlation, however, between the presence of eel-worm in the roots of hop and the false nettlehead condition—in fact, healthy plants showed on the average a

[1] Remarks in brackets [ ] inserted by the present writer.

greater number of worms per unit length of root than those suffering from nettlehead.

False nettlehead has long been known to occur in England, particularly in the variety *Fuggles* in the weald of Kent and Sussex, and is not to be confused with the true nettlehead or mosaic condition of the hop.[2] Salmon (29) (Fifth and Sixth Reports) says: "The cause of mosaic and nettlehead disease is probably to be attributed either to a virus or to an ultra-microscopic organism carried in the sap of the host-plant and transmitted from diseased to healthy plants by aphides. Investigations by Professor V. H. Blackman, F.R.S., and Miss Lacey are being made in order to obtain scientific evidence as to the cause of the disease."

In further reports on the trial of new varieties of hops by Salmon (Seventh and following Reports) emphasis is laid on the fact that the variety *Fuggles* is extremely, and indeed probably entirely, resistant to mosaic. "The continued resistance of the variety *Fuggles* to the mosaic disease is noteworthy. The two varieties, *Farnham Whitebine* and *Rodmersham Golding*, which are planted on either side of the *Fuggles*, have lost a considerable number of their hills during the last three years from attacks of the 'mosaic' disease, but not a single row of *Fuggles* has shown a trace of this disease." This resistance has since been shown [Salmon and Ware, 32; and also Mackenzie, Salmon, Ware and Williams, 22] to be complete, even after the most searching experiments. In a footnote to the Seventh Report Salmon again brings to our notice the susceptibility of this variety to the nettlehead disease, and he suggests that it is another disease of the virus type, but not as destructive to the hop gardens as the mosaic disease [Salmon and Ware (31), Brooks (3)].

Probably nettlehead occurs also in the European hop-fields, for Salmon (30), during a visit to the Bohemian hop districts, made a careful search for diseases of the virus type, more particularly for the mosaic disease, but found no trace of it. However, a disease, apparently of the virus type, was found in the Saaz district. "It was first seen in the experimental garden kept by Dr. Bauer at Saaz, who informed me that it was called the 'Krausel' disease, and that its cause was unknown. . . . . It appeared very similar to the nettlehead or so-called eel-worm disease, as it occurs on the *Fuggles* hop in the weald of Kent and Sussex, a disease which is now believed to be caused by a virus. The affected plants in Bohemia show the incurving of the margins of the leaves and also the shortening and partial sterility of the stems characteristic of the nettlehead disease, but the leaves showed in their tissues dead areas and holes, additional symptoms not observed, I believe, in the nettlehead disease. The disease in Bohemia—just as in England—affects a few plants in patches here and there in the garden, persisting in but not killing the plants. The same disease was seen

[2] Further reference to these diseases will be by the terms *nettlehead* (false nettlehead) and *mosaic* (true nettlehead).

in several gardens at Michelob and at Teschly. Herr Ing. F. Triska informed me that the disease had been known for thirty years or more" (Blattny, 2).

This appears to be the present state of our knowledge of nettlehead in hops, and although it has been suggested that it probably belongs to the virus group of diseases, this has not been *proved* a fact, and in the List of Common Names of British Plant Diseases (44), compiled by the Plant Pathology Sub-Committee of the British Mycological Society, and issued in 1929, nettlehead in hops is listed with the remark "cause unknown."

An examination of the symptoms and history of the so-called Tasmanian "take-all" suggests that it is very probably the nettlehead condition which has been recognized for so long in the Northern Hemisphere, although comparatively little is known about it. This conclusion is further strengthened by the results so far obtained from a study of the condition and described below.

PREVIOUS WORK ON "TAKE-ALL" IN HOPS IN AUSTRALIA.

Nicholls (26) has recorded the presence of an organism, *Plasmodiophora humuli*, on hops in Tasmania, and so far as I can ascertain he was dealing with hops showing the "take-all" symptoms, and which he ascribed to this organism. Nicholls, in correspondence, states: "When investigating the hop trouble in Tasmania about ten years ago, I found what I took to be a myxomycete and nothing else present in every case, and this opinion has been confirmed by later investigation. I was unable at the time to find any reference to it, until I came on one in Stevens (35), and I adopted the name *Plasmodiophora humuli* Kirk provisionally, as I found that in the hop fields in which I originally found the disease all the setts had been imported from New Zealand."

Sections of hop roots which had been prepared by Mr. Nicholls, and thought by him to show the presence of a myxomycete in the root, were sent to me, but after careful examination of them I failed to detect any organism in the root tissues.

P. humuli had previously been described as present in hops in New Zealand by Kirk (18). The tumours sometimes found on the roots and root-stocks of hop plants were thought by him to be due to a slime fungus, and he named the suspected parasite *P. humuli*. The photograph of these galls or tumours, which is reproduced in Kirk's paper, suggests that they were really examples of crown-galls caused by *Pseudomonas tumefaciens* Smith and Townsend. Moreover, I have had an opportunity of examining in detail spirit material of diseased hops belonging to the Department of Agriculture, Melbourne. The specimen was similar in appearance to the photograph in Kirk's paper, and it was labelled *P. humuli*. The anatomy of this specimen is almost certainly that of crown-gall, and microscopic examination did not reveal a myxomycete in the plant tissue. Again, Dr. Cunningham, mycologist, New Zealand, in correspondence with me,

states that "the name *Plasmodiophora humuli* was applied by Kirk to a disease, *not to a pathogen*, which is now believed to be due to a soil fungus (?), and enquiries made at the request of Dr. Butler, Imperial Bureau of Mycology, have failed to reveal any myxomycete."

It appears then that the name *Plasmodiophora humuli* Kirk is no longer valid. As the take-all condition of the hop in Tasmania does not even involve a malformation of the root-system, as was shown by Kirk's specimen, it is unfortunate that the name *P. humuli* was ever applied in connection with this condition.

Nicholls clearly distinguished between the "take-all" and crown-gall condition of hops. In the 1921-22 report he says, "Some of the hop roots received were affected with crown-gall or root-gall, due to bacteria of the type of *Pseudomonas tumefaciens*," but as Nicholls did not have access to Kirk's paper he could not realize that the name *P. humuli* Kirk was used in connection with a malformed root-system comparable to crown-gall, and so he applied it to a myxomycete which he considered to be the causal organism of "take-all," a condition not involving hypertrophy of the hop roots.

PRESENT INVESTIGATION.

ATTEMPTS TO ISOLATE A CAUSAL ORGANISM.

Considerable time was at first devoted to attempts to isolate a causal organism from affected hop setts. Material, as soon as collected in the field, was packed in sterilized containers and sent direct to Melbourne. Inevitably some delay occurred before this reached me, but on the whole the material arrived in fair condition. After thoroughly washing the plants with running water, portions of the various parts (young feeding roots, older lateral roots, rootstock, bine, and leaves) were cut out with sterile instruments. These were then washed in various antiseptic solutions, e.g., mercuric chloride, calcium hypochlorite, etc., and then in several changes of distilled water. Sections were next made and placed on various solid and liquid media, e.g., corn-meal agar, malt agar, hop agar, dung agar, soil agar, Brown's synthetic agar, potato agar etc., and incubated at 20–23°C.

Some organisms appeared rather regularly on those plates inoculated with subterranean parts of the plant, e.g., *Pythium* sp., *Fusarium* sp., and less frequently *Verticillium* sp. and *Rhizoctonia* sp. Several attempts to prove their pathogenicity failed,[3] and as the only parts of the hop plant which revealed fungal hyphae in the tissues on sectioning were those parts already showing advanced decay, e.g., older lateral roots, they are regarded as purely secondary parasites, or saprophytes, and are not considered to be related to the cause of the "take-all" condition.

[3] Hop seedlings and cuttings from setts were raised in the glass-house for this purpose.

Bacteriological technique was later employed in an attempt to isolate any bacterial pathogen that might be present. Material obtained from Tasmania at intervals between the months of September to February—that is, during the entire vegetative period of the hop—was treated in a variety of ways with a view to making bacterial isolations (Blattny, 2). Without entering into any great detail, a statement of the methods used may be of interest. Material as it came forward was washed in water, cut into sections, immersed in spirit, and then flamed or treated with mercuric chloride, and cut again with a sterilized knife, which was flamed after each incision. Then the tissue at the junction of the discoloured and apparently healthy region (if dealing with the decaying root-system), or entire fibrous roots and pieces of aerial bine, were pounded separately in sterile mortars with a definite amount of Lemco-peptone-salt broth. Sometimes this was allowed to stand in a covered sterile receptacle for 2–3 hours after pounding, or at other times the turbid liquid so obtained was plated out directly by the dilution method on to a series of Lemco-peptone agar plates, cellulose agar plates, etc., or inoculated into tubes of Lemco-peptone broth and incubated at varying temperatures ranging from 18–30°C. Roots (old and young), root-stock, bines, and leaves were all subjected to this treatment. After a few preliminary experiments, in which the pH of the medium was not controlled, attention was given to this factor, and media were employed having a pH over an approximate range of 5 to 8.

As a result of this treatment a number of bacterial colonies were isolated and maintained in pure culture. None of these on inoculation into the hop plant proved to be pathogenic to it, and a detailed examination of many of the forms so isolated showed the majority of them to be spore-forming organisms (supporting their non-pathogenic character), and many of them, too, were either obviously or after investigation found to be the more commonly occurring saprophytic forms (Mehta, 23).

HISTOLOGICAL EXAMINATION OF THE TISSUES OF AFFECTED HOPS.

Parallel with the attempt to isolate a causal organism, a detailed examination of the histology of the normal and infected plant was made, both by means of hand sections from living and fixed material, and also by microtome preparations. A number of fixatives were tried, but Flemming's (either weak or strong) proved to be the most useful. Difficulty was encountered in sectioning the woody parts of the plant satisfactorily, but this was met by immersing small cubes of the tissue, after fixation, in cellulose acetate for three weeks to one month (40). They were then removed, washed with acetone, and embedded in paraffin in the usual manner. The cubes after such treatment could be sectioned with comparative ease. Later similar cubes were placed for various periods of time in hydrofluoric acid, then thoroughly washed and embedded. Good sections were

also readily obtained after the use of this reagent. The chief stains employed for microtome sections were Heidenhain's iron-alum haematoxylin, Flemming's triple, safranin and licht grün, carbol-fuchsin, etc. Flemming's triple and Heidenhain's iron-alum haematoxylin gave the best results. Hand sections and entire portions of the young fibrous roots were stained with cotton-blue in lacto-phenol (Cook, 7), and mounted in lacto-phenol solution.

The bulk of the material used for this portion of the work was either fixed in the field during my visits to Tasmania or taken from diseased plants forwarded to me and subsequently grown in pots in the glasshouse of the botanical department.

A. Roots.

The finer hair-like fibrous roots appeared to be healthy, although not found in great abundance on infected plants. A great number of such roots were examined, and no organism was regularly observed which might be suspected as a factor directly contributing to the condition under examination. Occasionally an *Olpidium*-like parasite was met with in all stages of development in these rootlets, but this was too erratic in its occurrence to be considered seriously. Eel-worms, too, occurred abundantly, but Duffield (12) has recorded their equal distribution in healthy plants, so no significance can be attached to their presence in this connection, particularly as I also found them repeatedly in rootlets from healthy plants, although no attempt was made by me to compare the actual numbers present in roots from normal and abnormal setts.

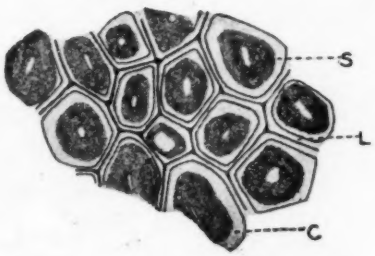

Figure 1. T.S. of cortical fibres from a normal hop-bine showing lignified and cellulose layers in their walls. × 625. L=lignified layer; C=cellulose layer; S=space between layers due to sectioning.

A transverse section of a fibrous root (approximately 4 mm. in diameter) showed three patches of primary xylem surrounded by about 1 mm. of secondary wood. Around this there was a broad zone of cortex with a superficially formed cork cambium. Cortex fibres, vertically of considerable length, together with phloem fibres, were arranged in a series of rays interspersed with parenchymatous

cells. The walls of these fibres are made up of two distinct layers, an outer lignified area, which responds to the usual lignin tests, and an inner, usually broader, cellulose portion. Very often these two layers separate from one another, leaving a space of varying volume between them.

In older roots considerable secondary wood was present, and decortication had occurred. Thrupp (38), while examining the phloem of cultivated hops affected with mosaic disease, noticed that some of the wood vessels were more or less plugged with a translucent, granular substance which would not take any of the ordinary stains, and, indeed, was very resistant to the reagents which he employed in attempting to elucidate its real nature. This plugging substance, although not previously recorded in *Humulus*, was found by him to be also

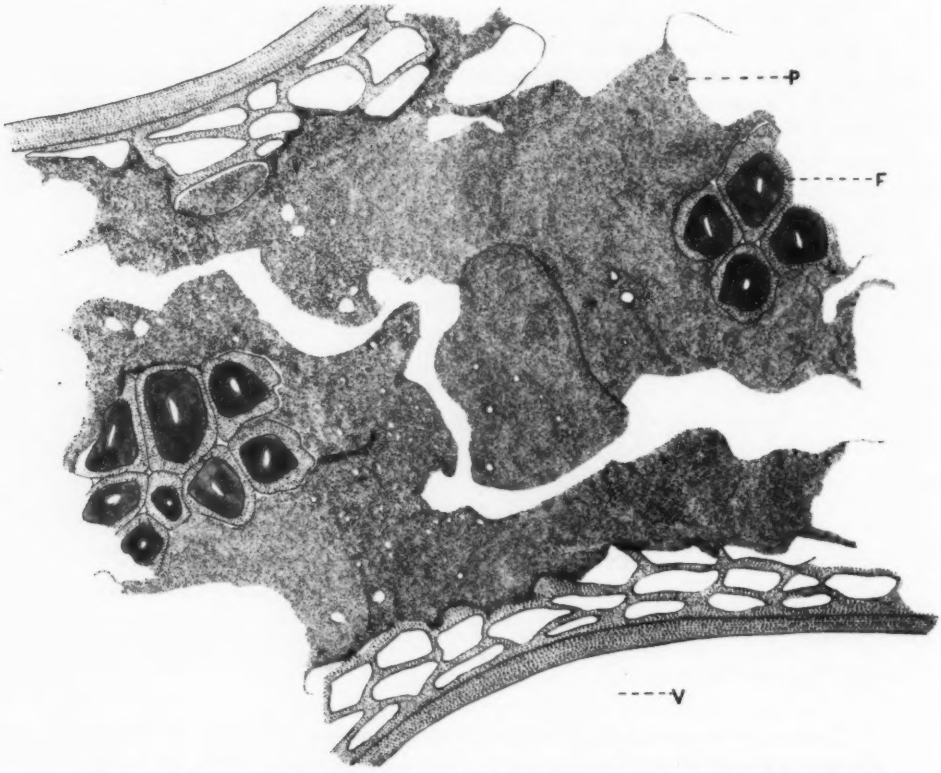

Figure 2. T.S. through a part of a root of a diseased hop showing one of the wood-fissures. "The plugging" substance has been formed in quantity and is filling up the space formed by the disintegration of the cells. The more resistant fibres are completely surrounded by this substance. × 625. V=wood vessel; P=plugging substance; F=wood fibres.

present in healthy plants. It has been encountered also in all the plants examined by me. In the larger roots from diseased plants a necrosis and disintegration of the tissues is evident, particularly in the phloem and cambial regions, but extending right into the wood on the one hand and the cortex, if present, on the other. In consequence it is very difficult to obtain hand sections through the older roots, if the disease is at all well advanced, as the various tissues tear or crumble apart. If a more or less complete section is obtained, however, irregular fissures are seen which may extend right through the root structure from the cortex to the innermost woody elements. The fissures in the wood run in both a radial and tangential direction, and are due to the disintegration of the thinner-walled cells surrounding the large wood-vessels. In unstained sections their boundaries are noticeable as a strong yellow-brown colouration is developed in their neighbourhood, owing to the exudation of great quantities of the plugging substance or gum referred to.

The gum occurs also in the cell lumen, both in the thinner-walled fibres, in medullary ray cells, and in the wood-vessels themselves.

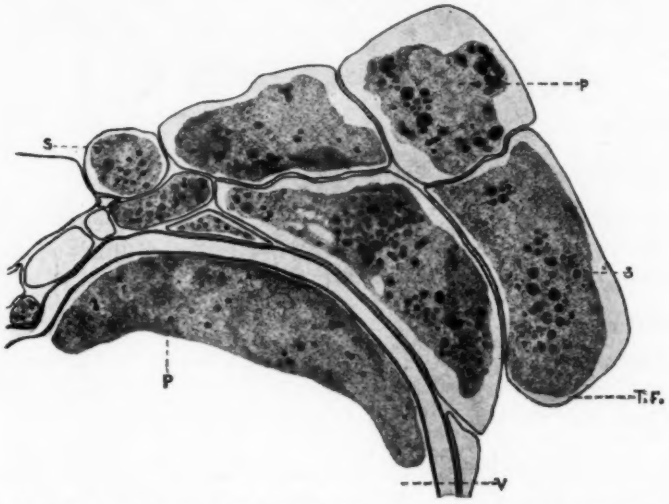

Figure 3. T.S. of the wood of a root of hop showing the deposits of gum in the cell cavity of the thinner-walled fibres and also in the lumen of the wood vessel. × 625. V= wood vessel; T.F.=thin-walled fibres; P=plugging gum; S=included starch grains.

The contents of some of these cells (starch grains, etc.) are often found embedded in it, and even the gum plugging the wood vessels is usually impregnated with similar inclusions. In preparations stained with Flemming's triple stain these included starch grains coloured with the gentian violet, and contrast

strongly with the gummy matrix, which remains uncoloured after this combination.

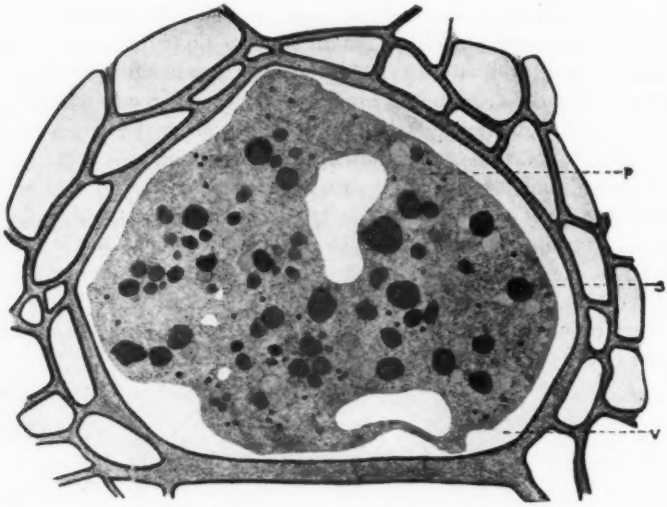

Figure 4. T.S. through a wood vessel from the hop root showing its bore practically filled with "gum" in which numerous starch grains are scattered. × 625. V=wood-vessel; P=plugging gum; S=starch grains embedded in gummy matrix.

Evidently some of the starch of the cells concerned in the formation of the "gum" has remained unchanged. Such grains become engulfed in it, and are carried along into the lumen of invaded cells (Swarbrick, 36).

B. Aerial Bine.

Transverse sections of the lower parts of the bine showed a necrosis in the phloem and cambial areas; here, too, disintegration of the cells seemed to occur, as radial and tangential fissures were present, occupied as before by the gummy deposit.

The wood, although many of the vessels were plugged as described by Thrupp, and the pith were not visibly affected. Text figure 6 shows in detail the cambial area of an infected bine a little distance above ground level.

Sections taken through the stem of the bine, about two feet from the ground and above, showed no marked histological characters not also shown by normal

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Figure 5. T.S. of lower part of hop-bine showing the gummy deposit in cambial and phloem regions and secondary lesions in cortex. × 625. W=xylem; PT.=pith; CA.=cambium; PH.=phloem; C=cortex; C.F.=cortex fibres; C.CA.=cork cambium; EP.=epidermis; P.=gummy plug.

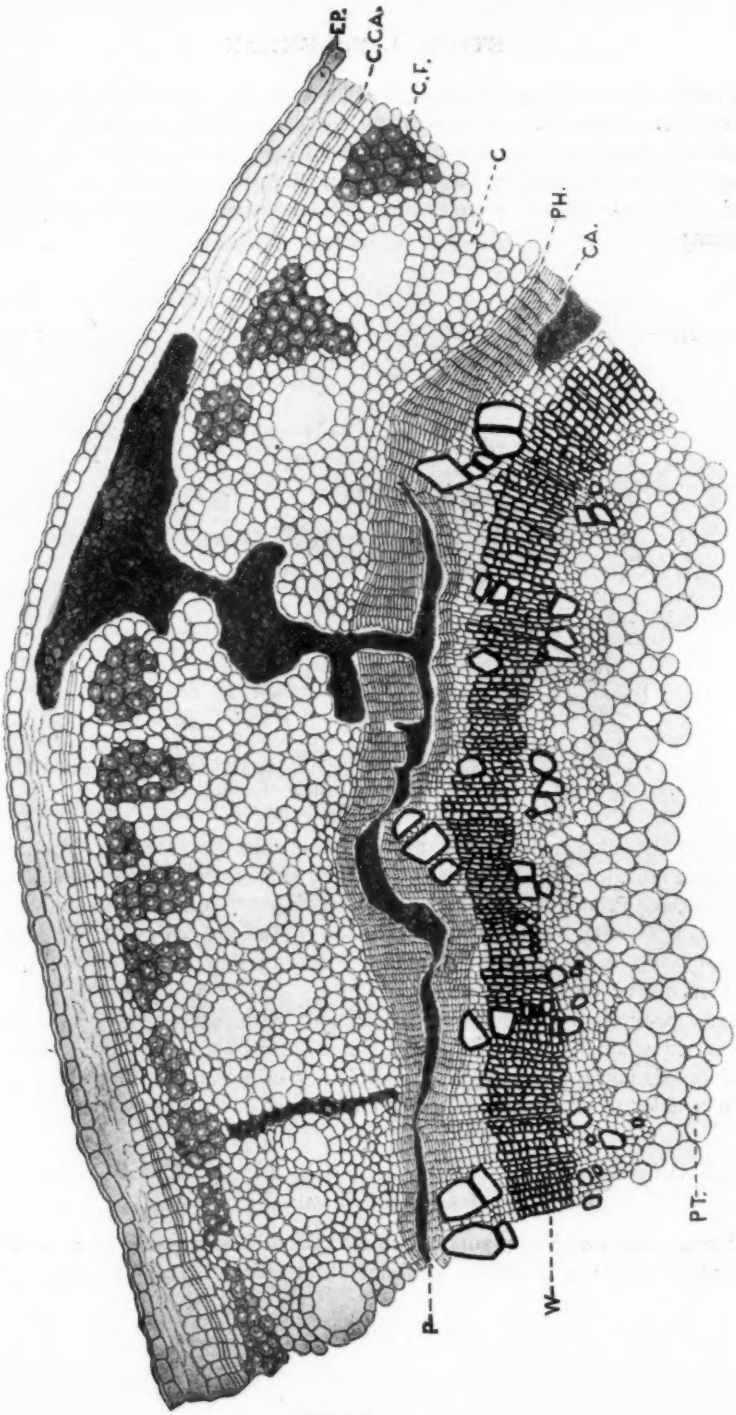

Figure 5.

tissue, with the exception that the phloem region was unhealthy in appearance, the cells seemed somewhat collapsed, and a distinct brown colouration was often developed. Some of the phloem cells showed inclusions which are probably similar to those described by Lacey (19), and interpreted by her as degenerated nuclei. Sections through petioles and leaf blade did not disclose any further symptoms.

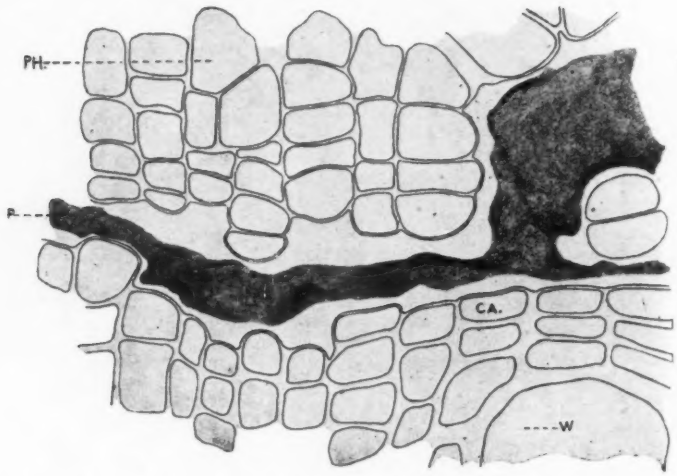

Figure 6. Cambial region of an infected bine close to ground level. × 625. W = wood-vessel; CA. = cambial segment; PH. = phloem; P. = gummy plug.

The results obtained support the suggestion that the "take-all" condition of Tasmanian hops is really a virus problem, and since the symptoms of the trouble are so similar to those described for "false nettlehead" in England, added to the fact that it is recognized as mostly a disease of the var. *Fuggles*, although var. *Golden Cluster* is not absolutely immune, it is further suggested that the two conditions are probably identical.

Some preliminary inoculation experiments with filtered juice have already been carried out, with the object of further substantiating this view. These have been delayed, however, owing to the difficulty of procuring material. I hope later to be able to continue these, together with some grafting experiments.

ISOLATION OF A PLASMODIAL FORM FROM TASMANIAN HOPS (*HUMULUS LUPULUS*, VAR. *FUGGLES*).

When examining hop plants showing "take-all" symptoms, on a number of occasions I found an organism, plasmodial in character, parasitizing the hop

plant, and although this is probably only a secondary parasite, as far as I know it has not been recorded before, and it is of interest from several points of view.

My attention was first directed to it when searching through sections of living material of "take-all" plants. It has been found both in and on root material, and also in the lower parts of the aerial bine. Sometimes a fairly large free plasmodium has been found creeping on the surface of the younger roots.

Occurrence in the Tissues of the Hop Plant.

The organism can penetrate into the hop root either by way of the root-hair or by direct penetration of the epidermis.

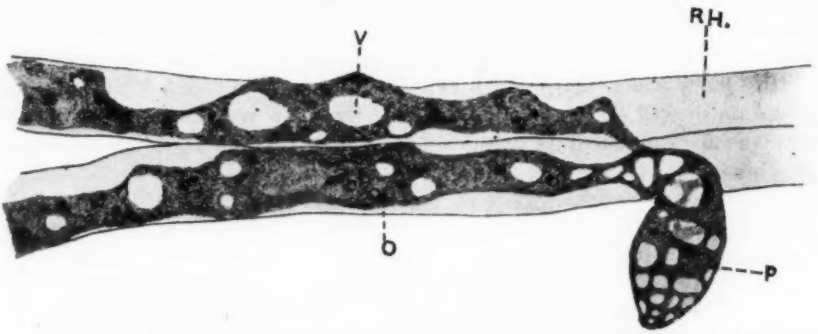

Figure 7. Plasmodium spreading over and through two adjacent root-hairs. × 625. P=plasmodium; RH=root hair; V=vacuole; O=oil drops. Drawn from living material.

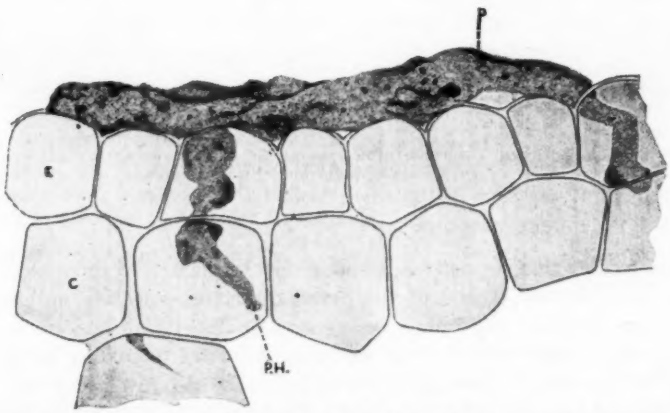

Figure 8. A plasmodium penetrating into a root directly through the epidermis. × 625. P=plasmodium; E=epidermal cell; C=cortex; PH=plasmodium in host. Drawn from material fixed in Flemming.

Sometimes the plasmodium spreads over and mats together adjacent root-hairs; finally entering into the hair it travels down it into the cortical tissue. If entrance is gained directly through the epidermis a single plasmodium may penetrate it at more than one point, and once in the root it can be either intracellular or intercellular in position, appearing readily to pass from cell to cell, and it does not excite any hypertrophic response of the host plant.

In preparations made from material which had previously been fixed in Flemming's solution, oil is conspicuous. It is difficult to see any nuclei distinctly in the plasmodia present in the hop tissue, although occasionally in a fortunate preparation they may be made out. This is not surprising, for it has been found, when dealing with the organism in culture, that careful and very quick fixation, in order to keep the plasmodium well expanded, is necessary before nuclear detail can be obtained, and then the nuclei, although numerous, are small.

Hop bines which had been grown in the glasshouse from "take-all" infected setts showed the plasmodium in their tissues as well as the roots. Text-figure 9 shows a plasmodium present in the cells of the inner cortical tissues of such a bine.

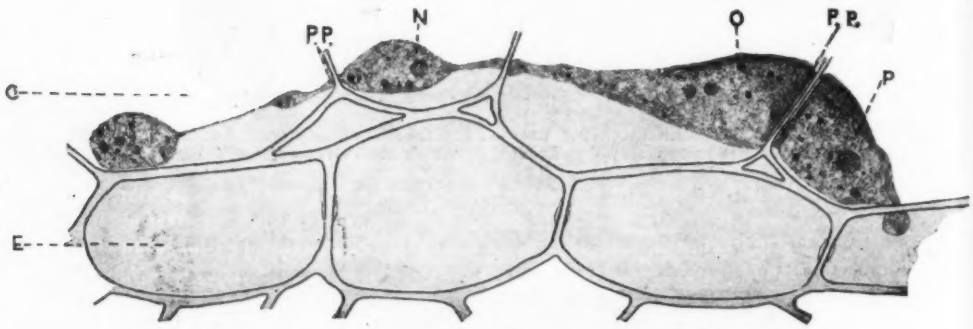

Figure 9. T.S. through a portion of an aerial hop-bine showing a plasmodium in its tissues. × 625. C=cortical cells; E=endodermis; P=plasmodium; O=oil; N=nucleus; PP=points of penetration from cell to cell.

It will be seen that the passage from one cell to the next may be accomplished by a fine protoplasmic strand of the plasmodium, necessitating only a minute pitting of the cell wall, or in some cases a greater dissolution may occur, for the plasmodium on either side of a cell wall may be broad in surface area. In the preparation figured penetration of the endodermis had just occurred. It can, however, extend into the vascular region. Text-figure 10 shows the woody tissue of the bine invaded by it.

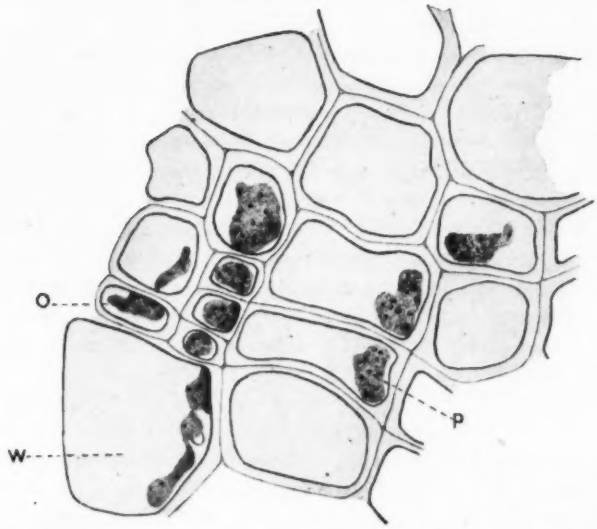

Figure 10. T.S. through the xylem of a hop-bine. × 625. W=wood vessels; P=plasmodium; O=oil.

It seems to pass through the wood-vessels by way of the pits on their walls. It is difficult to say whether the parasite has reached the base of the bines by way of root infections, or whether in watering or by the agency of rain, soil, in which the parasite is present, has been splashed on to the lower parts of the aerial bines and penetration has resulted in this way. Probably both can happen, for some of the sections examined showed the plasmodium in the outermost parts of the cortex, although actual penetration of the epidermis of the bine by a plasmodium has not been observed. It is not possible to trace the progression of the plasmodium through the hop tissues for any great distance by means of serial sections. These are very difficult to obtain, for infected material embedded in paraffin gave very poor sections. Cook (7) records the same difficulty in connection with roots infected with *Ligniera*. Hand-sections mounted in lacto-phenol had therefore to be mostly relied on.

The Organism in Culture.

Methods. Many attempts were made to cultivate this organism, and although the procedure was very varied no success was obtained at first; any surface sterilization seemed quite toxic to it, and if the material was unsterilized the plates and flasks were so quickly overrun with bacteria and fungi that they had to be discarded. However, later small setts suffering from "take-all" were sent

from Tasmania and successfully propagated in pots in the greenhouse. When the bines which appeared were still very small, in several instances the leaves commenced to wither at their margins. Some of these bines were severed and portions of the plant examined under the microscope. Plasmodia could be clearly seen in the tissues, and as this material was young and actively growing fresh efforts were made to isolate this form. Sections were placed on a semi-liquid hop agar infusion, in hay broth, in milk, in Knop's solution, Thornton and Smith's (37) solution, etc., but still with no better result. At the same time portions of the bine and leaves, after thorough washing in sterile distilled water, were put into sterile Petri dishes containing sterile water, and left in the laboratory at room temperature for some time. When these dishes were later examined a plasmodial form was found in quantity in the water and also in the hop tissue. Since ordinary mycological methods had not proved satisfactory for the propagation of this form the technique was altered and protozoological media were employed. Six to ten wheat grains were boiled to kill the embryo, and later soaked in 100 cc. of tap water in small Erlenmeyer flasks at 20–25°C. After from one to two days' incubation some of the flasks were inoculated with liquid from the original plates, and again incubated in the dark at 25°C.; other flasks, uninoculated, were incubated along with them as a control series. In this way the organism was grown and kept in the laboratory for some considerable time. Horlick's malted milk was also useful as a nutritive medium. The culture media were renewed at intervals by adding fresh wheat grains plus the water in which they had been incubated. In my experience this was a most satisfactory culture medium; and although transferences from such a culture were made to other media, e.g., Knop's solution, soil infusion, the plasmodia did not seem to thrive so well, no doubt owing to the fact that bacteria, although introduced into such flasks with the inoculum, would not be as abundant as in the wheat grain medium, and the osmotic concentration of the medium is also a factor to be considered. It is not possible to cultivate the organism in *pure* culture for bacteria, etc., are essential for its food, and even if a medium was devised to meet the requirements of the organism in this respect, the inoculation of it with the plasmodium would immediately introduce them into the culture, for it does not seem to be possible ever to free a single isolated plasmodium

EXPLANATION OF PLATE 1.

The microphotographs have been taken with a Zeiss photomicrographic apparatus.

Figure 1. A plasmodium of *Leptomyxa reticulata* Goodey var. *humuli* (nov. var.) photographed in the living condition. × 250. S=strands of the plasmodium; D=mass of defaecated material, etc., under which the plasmodium tends to hide if exposed to strong light; FP=ingested food particles; PPX=pseudopodia out of plane of focus.

Figure 2. Photomicrograph of a portion of a large plasmodium photographed in the living condition. × 300. EC=ectoplasm; EN=endoplasm; FPP=filar pseudopodia; LPP=lobose pseudopodia. (The colourless nature of the subject necessitated over-exposure of the negative.)

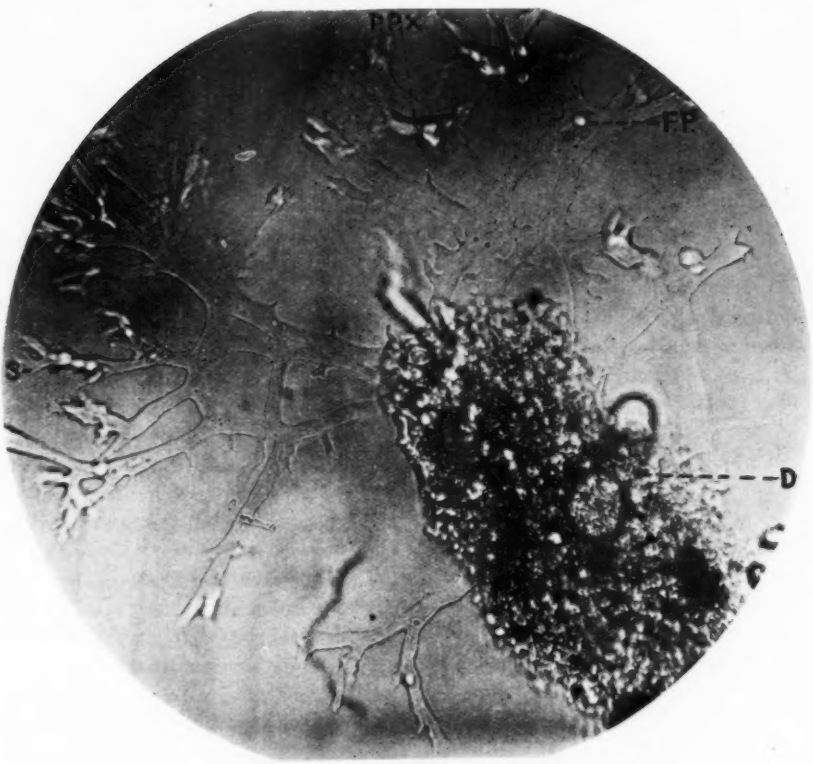

Fig. 1.

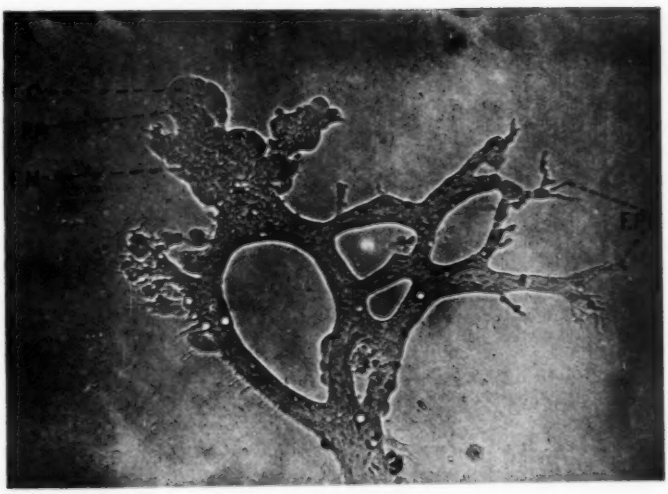

Fig. 2.

PLATE 1.

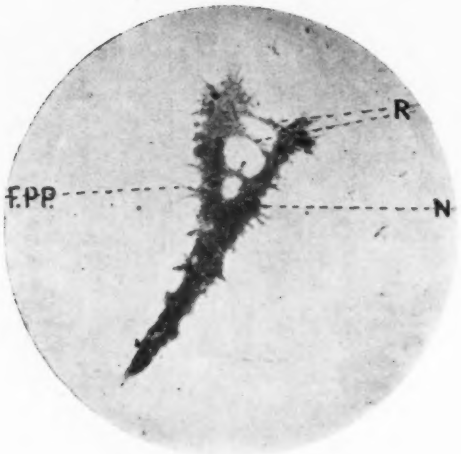

Fig. 3.

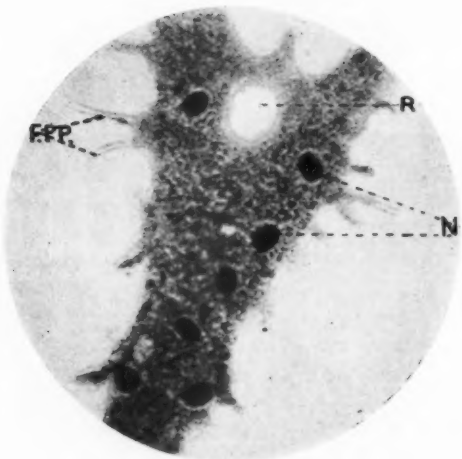

Fig. 4.

PLATE 2.

from adsorbed or otherwise adhering bacteria. Link, Jones and Taliaferro (21), working with *Plasmodiophora tabaci* Jones, record the use of "a pure culture of cysts and flagellates in Knop's solution obtained from a single plasmodium which had been isolated and transferred with a capillary pipette," probably meaning "*pure*" in that no other stage of the life-cycle of their organism but cysts and flagellates were present in the medium at the time of inoculation. This is a misleading use of the term, and should be avoided.

The plasmodial stage of the life-history of the organism was for some time the dominant one in the cultures under observation, although the conditions were varied as much as possible, in the hope that reproductive stages might be induced. Early in the progress of this work the method recommended by Miller (24) for obtaining the formation of sporangia by mycetozoa was followed, but naturally with no success.

For cytological investigation drops of the culture liquid were placed on a slide and covered with a supported cover-slip. Corrosive sublimate in alcohol was then run under the slip in such a way that the fixation of the organism could be watched under the microscope. This was followed by a solution of iodine in 70% alcohol. The washing with this liquid was continued until no decolourization of it occurred in the vicinity of the specimen. After further washing with alcohol the organisms were stained in various ways. Heidenhain's iron-alum haemotoxylin with various counter-stains was distinctly the most satisfactory. After clearing in clove-oil the specimens were mounted in balsam. Sometimes the organsm was fixed over osmic acid fumes, and the smears were allowed to dry on the slide; this gives greater ease in manipulation, but the detail of structure after this method is not so good. It was found later, however, that if some drops of the liquid from the culture flasks were placed on clean flamed slides and allowed to stand for some minutes, the plasmodia commenced to stream, soon becoming well expanded. In doing so they apparently applied themselves closely to the glass surface. After quick fixation with corrosive sublimate in this wet smear condition, the organisms remained attached, and it was perfectly safe to carry out the remaining stages of staining and mounting as for the dry smear method. If the fixatives were warmed before use better preparations were secured.

EXPLANATION OF PLATE 2.

Figure 3. Photomicrograph of a small plasmodium. This specimen had been fixed with Schaudinn's sublimate-alcohol and stained with iron-alum haematoxylin counterstained with eosin. × 300. N=nuclei showing deeply staining karyosome; R=reticulations of plasmodium; FPP=filar psedoupodia.

Figure 4. Photomicrograph of a portion of the same plasmodium as shown in figure 3. × 950. N=nuclei showing deeply staining karyosome; R=reticulations of plasmodium; FPP=filar pseudopodia.

Plasmodial Stage. The size of the plasmodia which have been observed varies over a wide range. (Plate 1, figures 1, 2; Plate 2, figure 3.) The smaller ones (containing from 12–20 nuclei) under medium illumination appear waxy and translucent, but when the amount of illumination is reduced they show a dense, little-vacuolated endoplasm, in which the nuclei are widely scattered, surrounded by conspicuous, exceedingly mobile ectoplasm (Plate 1, figure 2). The reticulations of the smaller plasmodia are few, but in the larger specimens they may be exceedingly numerous. (Plate 1, figure 1). Locomotion is accomplished by blunt, stout pseudopodia of the myxopodial type, i.e., when two come into contact they tend to fuse or anastomose (text-figure 11). (Plate 1, figures 1 and 2.) The protoplasm is highly miscible, and upon the fusion of many pseudopodia the meshed or net-worked plasmodium results (Calkins, 4).

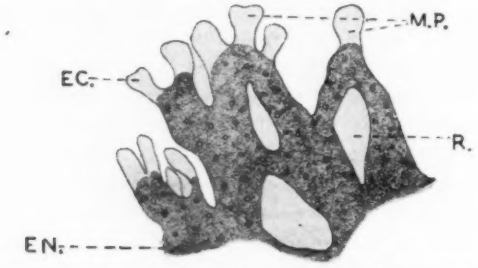

Figure 11. Portion of edge of a plasmodium showing the blunt locomotory myxopodia, drawn from a living specimen. × 625. EC=ectoplasm; EN=granular endoplasm; R=reticulations of plasmodium; MP=myxopodial pseudopodia.

Over the surface of this type of process a thick layer of ectoplasm is always evident. Sometimes portions of the plasmodia may take the form of exceedingly thin sheets of protoplasm spread out into a variety of shapes, but often enclosing no well-marked reticulations. In this state the finely-pointed pseudopodia are most evident. The protoplasm here is more quiescent. Locomotion in this particular direction has ceased (text figure 12).

This flat, film-like condition, however, may be changed quickly, and the more typical branched form established in its place. Contractile vacuoles are usually present in the protoplasm; they are small, and may be few or numerous, and in some instances they appear to be absent altogether; they contract very slowly. In the culture flasks the food-material of the plasmodia consisted mainly of bacteria, but larger forms, e.g., amoebae and their cysts, unicellular Chlorophyceae, etc., were often engulfed, and digested as in any typical amoeba. (Such forms were naturally present in the majority of the flasks, for the plant tissue from which the organism was obtained was only washed with successive changes of distilled water and then placed for an incubation period in dishes of sterile

water. Sub-culturing from this medium amoebae and flagellates were inoculated into the wheat flasks along with the organism under consideration. At a later stage individual plasmodia were isolated and cultivated apart from other protozoa.) A very characteristic feature of the larger plasmodia was the collection of undigested or defaecated material which appeared to remain attached to the posterior margin of the advancing organism for some time. (Plate 1, figure 1.)

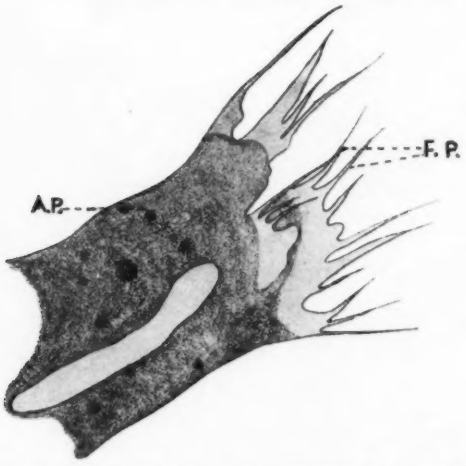

Figure 12. Portion of the edge of the same plasmodium as Figure 11, showing the filar pseudopodia round the more inactive part of the organism. FP=filar pseudopodia; AP=attenuated protoplasm.

Considerable difficulty was experienced in obtaining microphotographs of the plasmodia in the living condition, for well-expanded specimens, when subjected to the light necessary for the production of the photograph, immediately retracted themselves and became more or less "balled up" and useless for photographic or illustrative purposes. The retraction was almost instantaneous, suggesting a contractile movement, and resulted in the immediate disappearance of the reticulated type of plasmodium. The protoplasm aggregated itself into a compact mass, the surface of which was very irregular, and any one of these irregularities may become active pseudopodia when the right conditions are re-established, and by their movement the plasmodium is once again converted into the reticulated expanded state (text figure 13).

The interpolation of coloured glass filters[4] between the source of light and the specimen was found to be effectual in removing the irritant factor, and photographs of living plasmodia have thus been obtained (Plate 1, figures 1 and 2).

[4] The glass filter used was Wratten Screen B.

The large, fully-expanded plasmodium, figured in Plate 1, figure 1, was photographed in the living condition. The darker bodies visible along the boundaries of the meshes are not nuclei, but ingested food-material. It seemed characteristic of the organism that, when the liquid from the culture flasks was

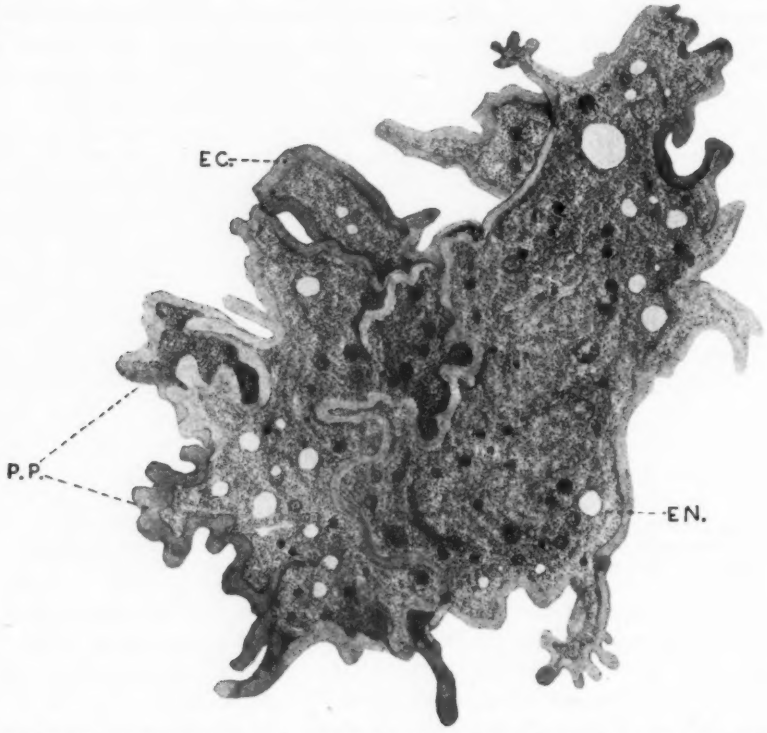

Figure 13. A plasmodium in the "balled-up" condition, drawn from a living specimen. × 60. EC=ectoplasm; EN= endoplasm; PP=potential pseudopodia.

first placed on the slides for microscopic examination, it remained hidden under relatively large masses of detritus as though light shy. If the slides were left undisturbed for a time the organism appeared round the margin of this mass, and finally it flowed out into the surrounding medium. To some extent these masses of foreign material represent the egested remnants of the food-material of the plasmodium. In the fully expanded state protoplasmic movement is extremely slow, and the organism has to be attentively watched before one sees that it is slowly but persistently changing its shape.

Nuclear Detail of the Plasmodia. Although permanent stained mounts were successfully made of smaller plasmodia without any great retraction taking place,

I did not succeed in obtaining any of the larger forms in an expanded state. Any of the fixatives and methods used resulted in "balling" of the larger forms, and when this occurred nuclear detail was lost; the organism in this condition is too dense for any cytological detail to be observed. Many of the smaller plasmodia, however, showed good detail after fixation and staining with iron-alum haematoxylin (Plate 2, figures 3 and 4). The plasmodia are multi-nucleated.

Text-figure 14 shows eight adjacent nuclei, and the drawing was made from a

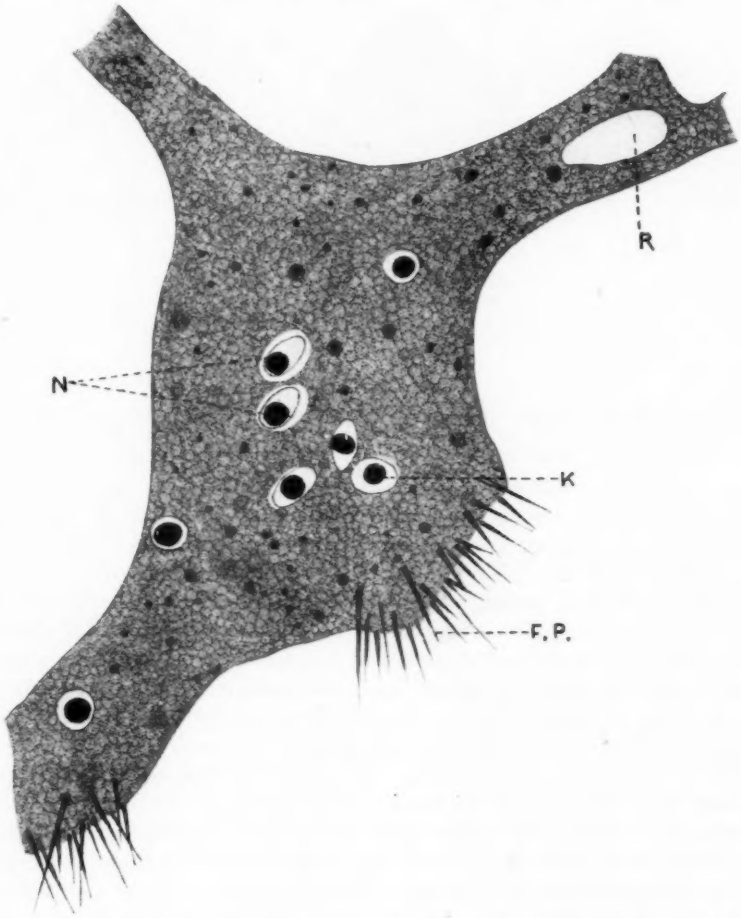

Figure 14. Portion of a plasmodium containing 20 nuclei, drawn from a fixed and stained preparation (iron-alum haematoxylin counter-stained with eosin). × 1,050. N= nuclei; K=karyosome; FP=filar pseudopodia; R=reticulation.

plasmodium containing twenty nuclei. They are seen to lie rather irregularly in the cytoplasm, and in some cases are in close proximity to one another.

They are vesicular bodies. A densely-staining, large karyosome occupies the centre of the nucleus, and the remaining chromatin material is scattered evenly between it and the nuclear membrane. The nuclei on the whole may be described as spindle-shaped, with some tendency towards a crescentic condition. Text-figure 15 indicates the variation of shape of neighbouring nuclei.

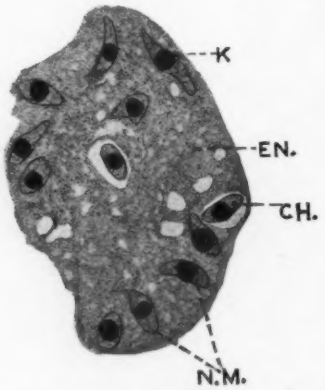

Figure 15. Portion of a fixed and stained plasmodium to show nuclear arrangement and structure. × 1,050. Twelve adjacent nuclei in a plasmodium containing approximately 160 nuclei. K=karyosome; NM=nuclear membrane; CH=chromatin material; EN=endoplasm.

This variation does not appear to be a result of fixation, as the plasmodium from which the figure was drawn was well expanded, and otherwise showed evidence of good fixation. Moreover, similar shapes were observed in living plasmodia. It is rather the result of protoplasmic streaming, which carries the nuclei along with it, and is no doubt responsible for a considerable amount of distortion. The heavier karyosome is often found at the posterior end of the nucleus; it probably lags behind in the nuclear sap. The large plasmodia have large numbers of nuclei, probably some hundreds. They are difficult to count, for the density of the protoplasm in some regions hides many of them from view in fixed mounts, and the ever-changing form of the living plasmodium renders counting impossible. The nuclei in these large forms are uniformly distributed throughout the endoplasm. Text-figure 16 shows a pseudopodium of a large plasmodium, with three nuclei crowded together, and situated almost at the junction of the ecto- and endoplasm.

All the nuclei were found to be in the resting stage, and although many preparations were made and carefully examined, the nuclei were never found in a state of division. It is well known that in the *Plasmodiophorales,* for example,

nuclear division is rarely found in preparations. Probably it takes place only occasionally, and even so is very quickly completed.

Multiplication. The plasmodia can multiply in culture by means of simple *plasmotomy.* In this process the parent form becomes separated into two or more daughter plasmodia, which remain attached to one another for varying periods of time by means of long, attenuated strands of protoplasm. These strands get thinner and thinner, and eventually usually break at the thinnest

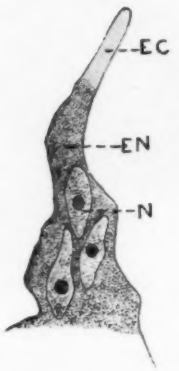

Figure 16. A pseudopodium of a large plasmodium showing the nuclei situated in the periphery of the endoplasm. EC=ectoplasm; EN=endoplasm; N=nuclei.

point. Although nuclear divisions were searched for at this stage in its life-history no success was obtained; each daughter segment was, however, always polynucleated. The reverse process, *plastogamy,* although recorded as happening in various protozoa, was never observed in my material. However, it is quite possible that it does occur, for one can only keep relatively few organisms under observation, and such a process could readily be missed.

Encysted State. (*a*) *Encystation.* Some of the flasks of wheat culture media, which had been inoculated on 17.4.28 with material from the dishes in which the plasmodia were first found developing away from the hop tissue (see above), and which had been incubated in the dark at 23–25°C., showed the development of large cyst-like structures. These were first observed on 16.5.28, but present in the same flasks were still numerous active plasmodia. The number of cysts increased as the contents of the flasks aged, but it was always possible to find a few small naked forms, most often in the "balled-up" condition. The cysts also vary in size from approximately 425–900μ, according to the size of the plasmodium from which they are formed. They vary in shape from completely spherical structures to an irregularly-outlined body. (Text-figure 17.)

They are completely surrounded by two delicate membranous coats, the inner

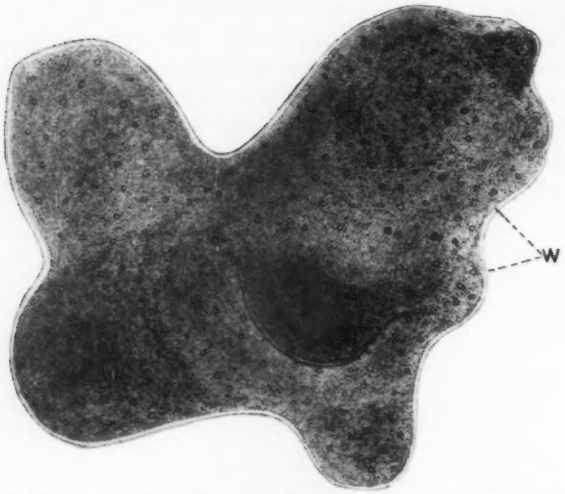

Figure 17. Resting cyst showing irregularity of outline, drawn from a living specimen. × 375. W = cyst walls.

of which, the endocyst, is transparent. Both it and the ectocyst are applied closely to the inner protoplasm. In this condition the contents appear to be very homogeneous. In living specimens sometimes small bodies may be seen scattered through the protoplasm; these, no doubt, are nuclei, for stained preparations show that the cysts are also multi-nucleate. (Text-figure 18.)

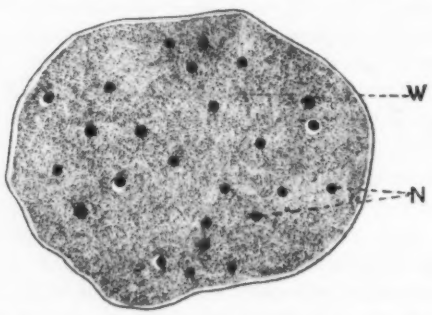

Figure 18. A cyst drawn from a stained specimen and showing its multinucleated character. × 375. W = cyst walls; N = nuclei.

The nuclei appear to be arranged regularly throughout the cyst. The karyosomes can be distinctly seen in such stained specimens. The rest of the nuclear structure is, however, very difficult to see. The cyst walls seem to be very per-

meable, as transference of the cysts from the watery medium in which they were grown to media of still comparatively dilute concentrations, e.g., Lemco-peptone broth, etc., causes plasmolysis to ensue immediately. The irregular contour of the larger cysts persists throughout their life, but some of the smaller are globular in shape. The ectocyst, so far as I have observed, remains thin, even in old cysts. In no case have I observed more than one endocyst enclosed by each ectocyst. Once encystation has been accomplished it was very difficult to induce excystation. This was eventually achieved by subculturing small quantities from the wheat grain media in which cysts were present into flasks containing very dilute Lemco-peptone broth (5 parts of this culture fluid to 100 parts of tap water), and incubating the flasks for short periods.

(*b*) *Excystation.* Excystation follows in a few hours after the cysts have been transplanted into the fresh medium. It is accomplished by the digestion of a portion of the cyst walls and the emergence of the contents in an apparently unaltered condition, and thus the plasmodial state is once more established. The endocyst wall is left behind inside the ectocyst.

Systematic Position.

When this organism was first isolated it was thought to be probably a member of the Mycetozoa, and, on that assumption, an extensive search was made for a sporing stage in its life-cycle, but without any success. When encystation and excystation were found to take place regularly under cultural conditions its affinities with the Protozoa were recognized, and more particularly with the section Proteomyxa.

This name was first given to a collection of primitive and little known forms by Hickson (16) in 1885, who says of the group: " . . . . Plasmodium formation occasionally occurs, but never leads to the production of a massive fructification, and . . . . encystment (or at any rate a resting stage at maturity) is a characteristic feature." A number of forms had been described, mostly very imperfectly, prior to this date, which, although members of the Sarcodina, did not seem to fit in with the rest of the Rhizopoda for various reasons, and the group Proteomyxa was established by Hickson to embrace them. The Proteomyxa correspond closely to Zopf's (43) group Monadineae. The two names were proposed simultaneously, but Proteomyxa has been retained in the literature. The majority of the earlier zoological writers, following on this, included in the Proteomyxa those forms which are now placed in the Plasmodiophorales. Cavers (5), in dealing with the Proteomyxa, states, "the division Proteomyxa is frankly admitted to be simply a sort of lumber-room for forms which it is hard to place under Rhizopoda or Flagellata, and which produce simple cysts for reproduction, not fructifications like Mycetozoa." Dobell (10) also emphasizes the chaotic nature of the group: "To-day it is hardly necessary to insist that Haeckel's 'Monera' (15) and similar groups ('Protamoebaea,' 'Proteomyxa,'

etc.) have no existence in nature, being mere scrapheaps for a number of unrelated organisms whose only common feature was that they were insufficiently studied and consequently misunderstood."

Since, even to-day, members of the group are so little known and somewhat varied in their characters, it is not surprising to find that there is still considerable confusion in the literature concerning their systematic position; no two works on Protozoa really agree in the placing of this group [cf. Minchin (25), Calkins (4), Doflein (11), Délage et Herouard (9)].

In 1914 Goodey (14) described three new forms which he had obtained from soil. All of them he grouped into the Proteomyxa, but he followed Minchin in regarding these forms as belonging to a sub-order of the Amoebaea, to which Minchin gave the name Reticulosa, and which he defined as amoeboid forms without a skeleton, and possessing filose or reticulose pseudopodia. The more recent classification of Calkins does not seem to the writer to be as useful as that proposed by Minchin. The conception of the Proteomyxa as possessing fine, radiate pseudopodia is not broad enough to include all its forms, and for this reason I prefer to follow Minchin and Goodey in regarding the Proteomyxa as a sub-order of the Amoeboea, broadly characterized by possessing, either or both, filose pseudopodia or myxopodia (Sandon, 33).

Two of the forms described by Goodey he placed in a new genus of the sub-order, viz., *Leptomyxa*. Both exhibited a plasmodial structure in the active condition, but he separated them into two distinct species, for in one—*Leptomyxa reticulata* Goodey—the pseudopodia anastomosed with one another, forming a reticulated plasmodium, while in the other—*Leptomyxa flabellata* Goodey—the pseudopodia remained distinct, never uniting to form fenestrae. When the organism found on the hop is compared with *Leptomyxa reticulata* the two forms are found to be essentially similar, and there is no doubt that the hop organism is correctly placed in this genus.

Goodey gives a critical review of the salient features of many of the forms allied to *Leptomyxa*, and which for long have been regarded as members of the Proteomyxa, including the genera *Arachnula* (6, 10), *Biomyxa* (20), *Amoeba dumetosa* (Penard, 27), etc.

Although most of these, but particularly *Arachnula*, show some points of resemblance to his genus *Leptomyxa*, Goodey considers that the differences are sufficiently great to warrant the establishment of this fresh genus. Comparing *Arachnula* and *Leptomyxa*, the main differences, according to Goodey, lie in the phenomena accompanying cystation in the two genera. Not only is this so, however, but the figures and description given by Dobell of *Arachnula impatiens* Cienk. suggest other differences which mark off *A. impatiens* definitely from the hop organism, and I consider also from *Leptomyxa reticulata* Goodey. The pseudopodia of *A. impatiens* are described as "very long and fine pseudopodia.

. . . . They may be thrown out at any point on the surface of the body, but are usually to be seen more or less definitely disposed in tufts at the ends or angles. The pseudopodia frequently branch, and are occasionally connected with one another by extremely delicate transverse threads like the pseudopodia of Foraminifera" (cf. Dobell, Plate 23, figures 1 and 2). The characteristic myxopodia and the coarse network of protoplasm arising from their fusion (Plate 1, figure 1) shown by the hop organism, and also found in *Leptomyxa*, is apparently absent in the genus *Arachnula*.

The size of the organisms composing the two genera also differs within very wide limits. Plate 23, figure, 2, of Dobell's paper represents a *large* specimen, magnification 400, and he states that "the longest individual which I have measured—in the form of an irregular band—was approximately 340μ from end to end (not including the pseudopodia), but this was an unusually long specimen." Goodey records *Leptomyxa reticulata* to a size of 1 mm., and some of the larger plasmodia I have found in culture exceeded this measurement. Again the reproductive cysts of *Arachnula* are 15–35μ in diameter, *rarely more;* unfortunately Goodey does not give the cyst measurements for *Leptomyxa reticulata*, but the cysts formed by my organism are on the average 500μ in diameter.

Cienkowski in 1876 described an organism which he styled "a fresh-water plasmodium." He did not give a more specific name to this form, for he apparently considered it to be a member of the Mycetozoa, and he doubtless thought that if given the right conditions it would pass over to a sporing stage, when a name might be more justly applied to it. At the same time he did observe the processes of encystation and excystation. The appearance of the hop organism was also, at first, exceedingly misleading in its resemblance to a plasmodium of a Myxomycete, and I searched for some time for evidence of its affinity with this group. Cienkowski's "fresh-water plasmodium" is in all probability a *Leptomyxa*, and the fact that he did not include this fresh-water form in his genus *Arachnula* lends further support to the distinction between the two genera.

The cysts formed by the hop organism in culture, although of the same general type (i.e., resting cysts as opposed to digestive cysts), appear to be much more irregular in shape, and on the average much larger, than those described for *L. reticulata*. Although Goodey does not include measurements of cysts, some idea of their size may be gained by an examination of his figures, of which the magnifications are given. The ectocyst in my form also, in so far as I have observed, never approaches the thickness attained in some cysts of *L. reticulata* and figured by Goodey. The latter organism has only been found in soil in England, and also in Russia (42), but never parasitizing a higher plant; in fact, none of the soil protozoa are known to be parasitic (Sandon, 33).

Nevertheless, taking all these points into consideration, I do not feel justified in separating the organism obtained from the hop into a new species for the distinction between the two existing species of *Leptomyxa*, viz., *L. reticulata* and

L. flabellata, is of a much higher order than the differences noted above. It is, in fact, such as almost to suggest a generic difference. In addition, I hesitate to add confusion to an already extremely involved group by multiplying ill-defined species, and so propose the name *Leptomyxa reticulata* Goodey var. *humuli* (nov. var.) for the organism under discussion. Summing up the systematic position, then, it is as follows:

Phylum: Protozoa.
Class: Sarcodina.
Sub-Class: Rhizopoda.
Order: Amoeboea.[5]
Sub-Order: Reticulosa (Proteomyxa).
Genus: *Leptomyxa* Goodey.
Species: *reticulata* (Goodey).
Variety: *humuli* (nov. var.).

DISCUSSION.

Jones (17) in America has recorded the isolation of an organism from tobacco plants manifesting mosaic symptoms, and to which he has given the name *Plasmodiophora tabaci*. This, as its name suggests, is a plasmodial form, but even if it proves to be a legitimate member of the Myxomycete group it is not well placed in the genus *Plasmodiophora*, for it apparently causes no hypertrophy of the host plant. Cook (8), in discussing the inter-relationships of the *Archimycetes*, refers to it as a doubtful species of this genus.

It was obtained in culture by placing small pieces of the plant tissue, which had been previously washed in 1 in 1,000 mercuric chloride solution, and then rinsed repeatedly with sterile water, in Knop's solution, and then incubating. In later experiments distilled water was found to be just as efficacious. The first organisms to appear were amoebae and flagellates, which later encysted. Each cyst gave rise to an amoeboid organism, and in turn a number of these fused to form a plasmodium. Jones demonstrates the sequence of these stages in the life-history by means of a diagram, but emphasizes that the only stage ever encountered in the tobacco plant was *the plasmodial stage*.

Pieces of tissue from other plants, e.g., tomato with mosaic, potato affected with leaf-roll—one presumes after similar treatment to the tobacco tissue, although this is not definitely stated—yielded amoebae and flagellates, differing somewhat in shape and size from those obtained from tobacco tissues. Amoebae and flagellates were also obtained from the intestine of aphids which had previously been fed on tobacco and tomato plants with mosaic symptoms, and on

[5] Schaeffer (34) in his monograph excludes any rhizopods with reticulose pseudopodia from the amoebas. He would place them in an order ranking equally with his Order Lobosa in which the amoebas are classed as of sub-order rank.

potato plants showing the symptoms of leaf-roll. These, however, apparently never passed over to the plasmodial condition.

I can state from my own experience in culturing the hop organism that any surface sterilization, unless used in such a manner or at such concentrations as to kill the plant tissue entirely, is *not adequate* to destroy many cysts or spores, belonging to diverse forms, which may be adhering to the surface of the host plant. There are, therefore, always present in the cultures many amoeboid and flagellate forms which have no connection with the life-history of *Leptomyxa*. The same thing must also apply to Jones's cultures of *Plasmodiophora tabaci*.

In such mixed cultures as Jones must necessarily have been working with, it seems almost impossible that the life-history could be followed with any certainty. Such observations could only be made by watching single individuals over a long period of time in hanging drop preparations.

Figures 63–66, Plate XXXVI, of Jones's paper are strongly reminiscent of some of the smaller specimens of *Leptomyxa reticulata* Goodey var. *humuli* nov. var. as I know it in culture. Figures 63 and 64 are referred to by him as exemplifying the formation of plasmodia by means of the fusion of long, stringy pseudopodia when they come into contact, and Jones states, "by this process hundreds of amoebae run together to form a huge plasmodium (figures 60–64)." The photomicrographs referred to might just as readily be interpreted as plasmodia which are separating into daughter plasmodia by means of plasmotomy. Further, the present ideas about plasmodial formation in the Myceotozoa is rather at variance with this statement (Wilson and Cadman, 41).

The contention that Jones has not adequately proved that the various amoebae and flagellates found in his culture flasks along with his plasmodium are stages in the life-history of one and the same organism is further strengthened in a paper by Link, Jones and Taliaferro (21), which appeared soon after the announcement of the isolation of *Plasmodiosphora tabaci* Jones from plants affected with mosaic symptoms. The object of the second paper was to settle the etiological role of this organism in tobacco mosaic.

In Table I, page 405, of this paper the number of cultures containing the organism after they had been inoculated with diseased and healthy plant tissues taken from various parts of the plant is set out. No indication is given, however, about the *stages* of the organism found in such cultures, and the question naturally arises: "Is the presence of amoebae and flagellates taken as the criterion of the presence of the organism, *Plasmodiophora tabaci* Jones, in these culture flasks?" That this is so is indicated by the large number of positive cultures obtained when healthy tissue, washed only with sterile water, was used as the inoculum. In one experiment out of a total of eleven culture flasks, ten showed the organism; in a second, of a total of five, two were positive (p. 405). When healthy tissue was washed, however, with 1 in 1,000 mercuric chloride solution, these numbers were much depressed, but, even then, out of a total of forty flasks

four contained the *organism* after incubation (as stated above, in no case was the *stage* in the life-history indicated).

Any healthy plant tissue taken from any host, and treated in the same way, would give such mixed cultures of amoebae and flagellates, and it is not sufficient to estimate the presence or absence of *P. tabaci* unless the plasmodial stage has been observed in the cultural flasks.

Further, Table II, page 406, includes a statement of the stage found in a number of the flasks (many of these are common to Table I), the contents of which were used as inocula into healthy plants, with a view to determining whether mosaic symptoms could be so induced in them. Although twenty-seven cultures are listed, *not one* of them contained the plasmodial stage. Similarly, Table III, p. 407, lists thirteen cultures, and again in none of them was the plasmodial organism observed. The authors say, "All of the inoculations were made with mixed cultures,[6] save one set made on July 30. This was made with a pure culture of cysts and flagellates in Knop's solution obtained from a single plasmodium which had been isolated and transferred with a capillary pipette."

If a plasmodium was transferred from a flask containing a mixed culture into a fresh culture solution by the use of a capillary pipette, no doubt the fresh culture solution would also be inoculated with some of the other members of the original medium, and these would multiply quickly under the fresh conditions. Therefore, the contents of this flask could in no way be regarded as a "pure" culture any more than the contents of the other flasks employed during their work. In addition, the number of the smaller forms would increase at a much more rapid rate than the original plasmodium, so that any inocula taken from this flask would still quite possibly not contain a plasmodial stage at all. In Table IV also the stages listed as occurring in the flasks did not include the plasmodial condition with the one exception of H E_3. The protozoa observed in this flask are given as plasmodia and flagellates. This, no doubt, is the culture referred to above, for on p. 409 is the following statement: "One of these (H E_3) was a pure culture of cysts and flagellates derived from a single plasmodium."

Finally, experiments were undertaken in order to see if *P. tabaci* had any filterable stage. Various kinds of filters were used, but in all cases the filtrates proved to be sterile, and *P. tabaci* did not appear; but to prove that the filtrates would support its growth, they were later inoculated with cultures of the organism, just at what stage in its development is not recorded. After an incubation period, an examination of the filtrates so treated disclosed again all stages but the plasmodium. The scarcity of the plasmodium in their experimental flasks is emphasized by these workers themselves, for special comment is made in the filtration experiments of the two cultures, 4C and 9C, obtained from diseased plants, and which contained plasmodia.

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[6] According to the tables amoebae, flagellates, cysts, "parasites", ciliates, were the forms present.

This evidence appears to me to weigh rather heavily against the idea that the life-history of *P. tabaci* has been correctly interpreted by Jones. If it has, why is it that the plasmodial stage was not obtained in the many cultures under observation in the later work? One explanation may be that the amoebae, flagellates, ciliates, etc., observed by Jones are contaminants derived from cysts, etc., which doubtless occur widely distributed over the surface of plants, both healthy and diseased, and have no real connection with the plasmodia noticed by him in his culture, the real cystic stage of which was probably not observed. It is possible that his plasmodium is a protomyxean-like organism closely related to *Leptomyxa reticulata,* occurring naturally in soil, and capable of entering and parasitizing a host plant already weakened by disease.

It is particularly interesting that the plasmodium (the only stage ever found by Jones in the plant itself) has been found associated with plants exhibiting certain virus symptoms, for *Leptomyxa reticulata* var. *humuli* has also been isolated from hop plants which it is thought are suffering from the attacks of a virus.

It is no doubt a secondary invader, and has no significance as a causative agent of this disease. It should be stressed that the protozoal forms reported by Jones and the present writer are quite distinct from the protozoan types found by other workers in virus-affected plants [Eckerson (13), etc.].

SUMMARY.

1. The symptoms of the disease known as "take-all" of hops in Tasmania are compared with those that characterize the "false nettlehead" condition of hops in England and Europe.

2. Previous work on this disease in Australia is reviewed.

3. A myxomycete, thought by Nicholls to be *Plasmodiophora humuli* Kirk and recorded by him as responsible for the "take-all" in hops in Tasmania, was not encountered in this investigation.

4. The name *Plasmodiophora humuli* Kirk is shown to be no longer a valid one. It was applied by Kirk to a crown-gall like condition of the hop, without an investigation of the causal organism.

5. An attempt to isolate a causal organism for this condition failed.

6. An account is given of the histological features of diseased hops.

7. "Take-all" in hops is thought to be due to a virus, and is probably identical with the "false nettlehead" observed for some years in English hop gardens.

8. A plasmodial form was observed in the tissues of *some* of the diseased plants. Its appearance in the host plant is described.

9. This organism was isolated, and considerable attention was given to it in culture. It was found to be a protozoan organism, showing protomyxean characters, and it was identified as *Leptomyxa reticulata* Goodey var. *humuli* (nov. var).

10. This is the first record of the genus *Leptomyxa* Goodey for Australia. It has been recorded as occasionally present in soil in England and Russia (?).

11. *Leptomyxa reticulata* var. *humuli* is probably the first soil protozoan to be found parasitic on the higher plant.

12. The vegetative condition of the organism is described both from living and fixed specimens.

13. The method of multiplication and cystation has been followed.

14. A discussion of the systematic position of *Leptomyxa reticulata* var. *humuli* is included, and a comparison is made with some other closely related organisms, such as *Arachnula* Cienk., and Cienkowski's "fresh-water plasmodium."

15. A critical review of Jones's work on *Plasmodiophora tabaci* is given, and the suggestion is put forward that *P. tabaci* Jones and *Leptomyxa reticulata* Goodey may prove to be identical or closely allied organisms.

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STUDIES ON INFECTION BY AND RESISTANCE TO THE PREISZ-NOCARD BACILLUS.

1. THE PREPARATION AND STANDARDIZATION OF BACTERIAL EMULSIONS.[1]

by

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In studying immunity to bacterial diseases it is essential that some reliable and standard method for measuring the pathogenicity of the bacterial emulsion should be available. The pathogenicity will vary with the conditions under which the micro-organism has been kept following isolation from the animal body as well as the nature of the culture medium, the fluid used for suspension and other conditions, all of which can be brought more or less under control. The infectivity of some bacteria is so great that the minimum infecting dose may possibly be so small as to be represented by a single viable micro-organism or some small multiple thereof. There are difficulties in measuring so small a dose with any degree of certainty. These difficulties are greater when working with those bacteria that are pellicle-forming under conditions of artificial cultivation in liquid media. Such bacteria tend to hang together in clumps, and even after being separated from one another by mechanical means, often reclump after a very short time.

Various methods have been devised from time to time for the purpose of eliminating clumps in bacterial suspensions and for estimating the number of bacteria present. Probably the tubercle bacillus has been found to be one of the most difficult micro-organisms with which to obtain satisfactory suspensions. Suspensions of this micro-organism have been made by grinding up the growth with a mortar and pestle, gradually adding saline or phosphate solution, gently centrifuging the suspension for a few minutes to remove clumps, and standardizing the suspension by counting the number of bacilli present.

The present communication records observations made on the standardization of suspensions of the Preisz-Nocard bacillus, the causal organism of

[1] These studies are being carried out as part of a larger scheme for the investigation of caseous lymph-adenitis in sheep sponsored by the Commonwealth Council for Scientific and Industrial Research.

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caseous lymph-adenitis of sheep. It is believed that the methods evolved may be applied to other types of bacteria, but more particularly those possessing clump-forming potentialities.

PRELIMINARY OBSERVATIONS.

Methods of Artificial Cultivation. The first essential is to find an artificial culture medium which favours the retention of the pathogenicity of the micro-organism. We have found that the Preisz-Nocard bacillus can be isolated from lesions with ease on Loeffler's inspissated blood serum, blood agar, or hormone agar. We have found Loeffler's blood serum the most satisfactory for obtaining primary growth. This is then subcultured on hormone agar, and if found to be pure tubes of alkaline egg medium are inoculated for the purpose of stock cultures. These are incubated for twenty-four hours at 37°C., and then kept at room temperature. The stock cultures are subcultured at intervals of from six to eight weeks. A tube of hormone agar is inoculated from the stock culture and incubated for 48 hours. This growth is used to inoculate heavily a fresh tube of alkaline egg medium. By this method the organism has been found to retain its toxogenicity and pathogenicity for a period of two years or more. If the stock cultures are subcultured at longer intervals, toxogenicity and pathogenicity are more quickly lost.

The Preisz-Nocard bacillus belongs to the genus Corynebacterium. In films of the caseo-purulent material from lesions in sheep the bacillus is found to be Gram positive and somewhat pleomorphic, club-shaped, ovoid, filamentous and branching forms being observed. On artificial culture media the morphology is more constant, ovoid forms predominating, and measuring about $0{\cdot}5\mu$ broad by $1–1{\cdot}5\mu$ long. Single forms are not frequent, the bacilli arranging themselves mainly in groups. In liquid media pellicle formation is almost constant, but the pellicle may easily fall, giving a granular deposit with clear supernatant fluid. On solid media the growth, however luxuriant, is always somewhat dry, and when washed off comes away in flakes or in a granular state.

Suspensions. For the purpose of making a bacterial suspension a subculture is made on hormone agar and incubated for 24 hours. The growth of this first subculture is not usually very luxuriant. A second subculture gives a luxuriant growth. From this growth the suspensions are made. Some preliminary attempts were made with the growth obtained in hormone broth collected by centrifugation in a tube stoppered at the distal end by a rubber stopper. The supernatant broth was discarded, and the bacterial growth collected on the cork was ground up in an agate mortar and suspended by adding normal saline a little at a time. The suspension was filtered through a double thickness of Whatman No. 1 filter paper, but clumps were found to be present in the filtrate.

Whatman No. 44 removed the clumps from the suspension, but removed most of the micro-organisms. No greater success was achieved by using M/15 sodium phosphate solution for suspending the organism, nor after vigorous shaking with glass beads in a mechanical shaker for two hours. The same poor results were obtained with growth whether from solid or liquid media.

Attempts were made to obtain a more uniform growth in broth, i.e., to prevent the formation of pellicle and granular growth. Extraction thimbles were suspended in flasks of broth, and the fluid inside the thimbles inoculated. After incubation it was found that the growth diffusing into the surrounding medium was still somewhat granular.

Unsuccessful attempts were also made to obtain bacterial suspensions from the purulent material obtained directly from lesions in sheep. The removal of the cells and cellular detritus by centrifugation was found to remove also the majority of the bacilli. Further attempts were made by inoculating guinea-pigs intraperitoneally with cultures of the organism, and the contents of the cavity were collected in citrated saline. The cells were removed by centrifugation, but the supernatant fluid contained few or no micro-organisms.

As these observations gave no promise of success, further attempts were made to obtain a more uniform growth in broth by incorporating in the medium either pancreatic extract or bile salt. No encouraging results were obtained with the pancreatic extract, but by lowering the surface tension of the medium with bile salt pellicle-formation was prevented, although the growth was still somewhat granular. By gently shaking the growth in the bile salt broth an even suspension was obtained. A concentration of 0·12% of bile salt was found to give the best results. Lower concentrations allowed the development of a pellicle, and higher concentrations had an inhibiting effect on growth. Increasing the colloids in the bile salt broth by the addition of serum or gelatin helped to make the suspensions more permanent, but some clumping of the micro-organisms still remained. The surface tension of the broth was also lowered by saponin and caprylic alcohol, but no satisfactory results were obtained by these means.

Fairly satisfactory results were obtained by growing the bacillus in hormone broth, with the addition of 0·12% of bile salt, gently centrifuging for a short time to throw down the clumps, collecting the remaining micro-organisms in the supernatant fluid by vigorous centrifugation, and resuspending the deposit in M/15 sodium phosphate (pH 8·7) containing 0·12% of bile salt. It was found, however, that the solution of sodium phosphate and bile salt developed an opacity. This was almost completely prevented by using a specially purified bile salt kindly supplied to us by the late Professor Brailsford Robertson. It was found, however, that the mixture of sodium phosphate and bile salt was not entirely satisfactory, as an opalescence often developed after sterilization by heating. Many other minor difficulties were encountered in

subsequent work. One observation is probably of sufficient interest to merit mention. It was found that the reaction of the fluid in which the bacilli were suspended was of prime importance. A solution of bile salt and sodium chloride, carefully adjusted to pH 8·7 with sodium carbonate, would develop some opalescence after sterilization by heat. If the solution were cleared and sterilized by passing through a Seitz filter the reaction would, however, become more acid. The following is a record of one such observation:

Solution before filtration	pH 8.6
First 20 cc. through filter	pH 7.3
Next 100 cc. through filter	pH 7.9

It was found that the alteration in reaction after filtration rendered the solution unfit for use.

Finally the following method was found to give consistently good results:

Method of Making Bacterial Suspension.

A subculture is made on a hormone agar slant from the alkaline egg medium stock. After 24 hours' incubation at 37°C. the growth is again subcultured on hormone agar. The 24 hours' growth on the second subculture is washed off in bile saline prepared as follows:

All glassware is carefully prepared to ensure perfect cleanliness and freedom from any trace of free acid or alkali. A 0·1% solution of sodium chloride is made and adjusted to pH 8·9 with sodium carbonate. It is autoclaved at 115°C. for 20 minutes. Sterile distilled water is added to make up any loss. The bile salt is prepared in a 10% solution and sterilized at 115°C. This is kept as a stock solution. Bile salt is added to the sterile 0·1% saline solution to give a concentration of 0·12%. The final reaction is adjusted, if necessary, to pH 8·9 by adding sterile sodium carbonate solution.

The bacterial growth washed off into this bile salt saline is placed in small sterile bottles containing glass beads, and with a rubber stopper. The emulsion is shaken in a mechanical shaker for fifteen minutes and then centrifuged. The supernatant fluid is discarded and fresh bile salt saline added. It is again shaken for fifteen minutes and then centrifuged. The process is repeated, so that, in all, the emulsion is shaken three times and centrifuged twice. The final suspension is transferred to a centrifuge tube, and centrifuged at low speed for about five minutes to thrown down any clumps which have remained unbroken. The emulsion is then filtered through a sterile Postlip filter paper, No. 6335. When the major clumps are removed by the centrifuge before filtration is performed the filtrate contains the major portion of the emulsion, little being lost on the filter paper. In this way the emulsion is obtained free from clumps. A microscopic examination of the emulsion is made, and if any clumps consisting of three, four, or more bacilli are found it is discarded. Provided that there

has been no increase in hydrogen ions in the solution, perfectly consistent results are obtained.

Estimation of the Bacterial Content.

It is generally agreed that the accurate estimation of the number of bacteria in a suspension requires a very special technique, and that many pitfalls may be encountered. The difficulties are greater when the micro-organism to be counted will not suspend evenly in the dispersion media usually employed. With the evolution of the Helbe counting chamber micro-organisms could be counted in the stained condition under open illumination by the means of a $\frac{1}{12}$-inch objective. Even with this instrument a large experimental error may occur. Wilson (1), in a study on the viable bacteria in young cultures, adapted the Helbe counting chamber to dark-ground illumination by using a slide of such thickness that the distance between the lower surface of the chamber and the upper surface of the condenser was between 0·9 mm. and 1·1 mm. With this instrument he obtained accurate results, with a small experimental error, when the preliminary dilution of the suspension was made with great care.

Unfortunately, an instrument such as Wilson used has not been available in these studies up to the present. Many methods have been tried by us, but so far none has given consistently accurate results. Although it has been desirable to obtain absolute accuracy, for our purposes this was not essential. The most essential point was to obtain consistent results, even if the absolute number of bacteria present per volume could not be accurately stated.

Wilson has shown in his study that the most accurate results are obtained when dilution of a suspension is made by the drop method. He calibrated his pipettes with an Imperial Standard wire gauge, but found that inaccurate results were obtained unless these pipettes were subsequently tested by a screw micrometer measuring to 0·01 mm. The drop values were estimated by gravimetric methods. He found that there was no appreciable difference in the drop values of suspensions ranging in thickness from 500 million to 10,000 million micro-organisms per cubic centimetre, apparently the weight of the micro-organisms just sufficing to counterbalance the increasing viscosity of the liquid. He states that a small number of bacteria remains adherent to the walls of the pipettes, but that the error resulting does not appear to be greater than 1%.

We had gone some distance in attempting to estimate the bacterial content of our suspensions before Wilson's paper was consulted. We had not appreciated the great error that might occur as a result of the property of bacteria to cling to the glass walls of a pipette, even when it is scrupulously clean. It may be interesting to illustrate the results that may be obtained when this source of error is neglected.

An emulsion of the Preisz-Nocard bacillus was made in bile salt saline, as outlined above. This was estimated to contain 1046·5 million bacilli per cc.,

and was diluted with bile salt saline by means of a 1 cc. pipette graduated in 1/100ths to the tip. In the one case the dilutions were made by using a separate, clean, dry pipette for each transfer, and in the other by using the same pipette throughout. In making the transfers the dilutions were mixed by drawing the fluid in and out of the pipette twelve times. The following are the details of the actual steps and quantities used in making the dilutions:

P.E.=Primary emulsion=1,046,500,000 bacilli per cc.

(a) 2 cc. P.E. diluted	to 2·09 cc.	= 1,000 million per cc.
(b) 0·1 cc. of (a)	to 9·9 cc. saline	= 10 „ „ „
(c) 0·1 cc. of (b)	to „ „	= 100,000 per cc.
(d) 0·1 cc. of (c)	to „ „	= 1,000 „ „
(e) 0·1 cc. of (d)	to „ „	= 10 „ „
(f) 1·0 cc. of (e)	to 9·0 cc. „	= 1 „ „
(g) 1·0 cc. of (f)	to „ „	= $\frac{1}{10}$ per cc.
(h) 1·0 cc. of (g)	to „ „	= $\frac{1}{100}$ per cc.
(i) 1·0 cc. of (h)	to „ „	= $\frac{1}{1000}$ per cc.

These emulsions prepared by the two methods of dilution were used for inoculating guinea-pigs. Two guinea-pigs were used for each dose of the emulsions respectively. Doses representing 10 micro-organisms down to 1/10,000 of a micro-organism were given in the case of the dilutions prepared with separate pipettes. Doses representing 1/100 of a micro-organism to 1/10,000 of a micro-organism were given in the case of the dilutions prepared by using the same pipette throughout. In the first case none of the guinea-pigs developed a lesion, and in the second case all the guinea-pigs developed lesions, except one of the pair receiving the lowest dose.

The result of this experiment illustrates a carry-over from tube to tube, due to the micro-organisms clinging to the sides of the pipette, and gradually being released as the pipette is washed out in subsequent volumes of bile salt saline. Unfortunately the doses were not high enough in the one case to produce infection in the guinea-pigs, in spite of the highest dose being calculated to contain 100,000 more bacilli than the lowest infecting dose in the other case.

This clinging can be overcome to some extent by using pipettes coated with paraffin wax, but cannot be entirely eliminated. It was thought that the Preisz-Nocard bacillus might be more "sticky" than other types of bacteria, but the same phenomenon has been observed in making dilutions of an emulsion of *B. typhosus*, as the following experiment illustrates.

An emulsion of a 24-hours' old subculture of *B. typhosus* on nutrient agar was made in 0·9% sodium chloride solution. The emulsion was estimated to contain 1,830 million bacilli per cc. It was broken down by making a series of 1 : 10 dilutions, and then adjusting to a whole number, viz., 400 bacilli per cc. The dilutions were made by three methods: (a) by using the same pipette throughout, with twelve washings on each transfer; (b) by using a new pipette for each transfer, each pipette being washed out twelve times; and (c) by using a pipette lined by paraffin, but in all other respects the method being the

same as (a). Portions of the final dilutions were plated, with the following results:

Method (a) gave 2,305 colonies per cc.
" (b) " 359 " " "
" (c) " 886 " " "

The actual figures are not regarded as being accurate. There may have been, and probably were, more than 1,830 million bacilli in the primary emulsion. The figures are, however, of value for comparative purposes. The large error obtained by method (a) is apparent. Allowing for the presence of non-viable bacilli, method (b) appears to give more accurate results than are probably real. Method (c) indicates that even with a pipette coated by paraffin a definite carry-over of micro-organisms occurs.

These results suggested that the method of dilution employed in making routine bacterial counts of milk might lead to gross error. Some preliminary observations, however, have indicated that there is little or no carry-over of bacteria in this medium if the pipette is washed out from fifteen to twenty times between each transfer.

Accurate estimations of the number of bacteria in our emulsions has not so far been possible by the means at our disposal. It is believed that Wilson's technique, using dark-ground illumination, would give accurate results.

After trying various methods, we believe that approximate estimates of the number of bacteria present have been arrived at by making direct counts against sheep's red blood cells. Oxalated sheep's blood, formalinized by adding 0·1 cc. of 10% formalin in saline to 5 cc. of blood, has been used in this method. The bile saline used for making the emulsion has contained 0·5% sodium chloride and 0·12% bile salt brought to pH 8·9. Equal quantities of bacillary emulsion and red cells have been mixed together, and films prepared in the usual way and stained by Jenner's stain. Numerous counts have been made and a standard established. It is realized that the standard established is only an approximate estimate of the actual number. In order to compare one emulsion with another an opacity standard has been prepared, and comparison carried out in the Klett bio-comparator. Barium sulphate suspensions and milk suspensions prepared from dried, skimmed milk have been tried as standard suspensions without satisfactory results. Very satisfactory results have been obtained by using a nineteen months old formalinized emulsion of *B. oedematiens*. This was a strong emulsion of the bacilli preserved in 0·5% formalin in normal saline. A 1% dilution of this emulsion prepared in a volumetric flask gave a good, stable emulsion. Formalin was added to the emulsion to give a final concentration of 0·05%. By the use of this standard emulsion accurate comparisons between different emulsions of the Preisz-Nocard bacillus can be made.

In testing out these standardized emulsions of the Preisz-Nocard bacillus

by injection of the guinea-pig irregular results are obtained. Sometimes doses calculated to contain 100 bacilli, possibly only from 30–50% being viable, will infect a guinea-pig, whereas at other times ten times this dose will fail to infect.

An attempt to determine the number of viable bacilli present in these emulsions by cultural methods has not given very satisfactory results, as the culture medium used does not appear to favour the development of single colonies, although luxuriant growth is obtained by ordinary massive inoculations. In these studies hormone broth and agar have been used extensively. The hormone broth is made by taking 5 pounds of minced ox heart and infusing this in 3,000 cc. of water overnight. A broth culture of *B. coli* is then added, and the whole incubated for 24 hours at 37°C. Three beaten eggs are added, and the bulk made up to 5,000 cc. Peptone 2%, sodium chloride 0·5%, and gelatin 1% are added, and the whole boiled for 15 minutes. It is allowed to stand until cool, and the supernatant fluid siphoned off. Further clearing may be necessary, when the white of an egg is added for each litre of fluid. The reaction is adjusted to pH 7·6, and sterilization is done in flowing steam on three consecutive days. When used for plating purposes 2% agar and 5% sterile sheep serum are added.

Results have indicated that usually only about 30%, or less, of the bacteria present in these emulsions are viable on artificial culture media, but that their pathogenicity may be seriously lowered. Possibly the bile salt, in the absence of any protecting colloids, is responsible for this injury and loss of pathogenicity. As the manipulations take several hours to complete, injury to and destruction of bacilli must occur, and possibly these injurious factors are augmented by any rise in temperature. As the shade temperature is very variable in this climate, and as temperature during manipulation is the only factor not brought definitely under control, it seems possible that this variation in temperature is one of the factors responsible for the irregular results obtained in the attempts to determine the minimum infecting dose of the emulsions.

An attempt to find a satisfactory solution of this difficulty is engaging our attention at the present time.

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STUDIES IN CENTRE OF GRAVITY CHANGES

1. A NEW METHOD FOR FINDING THE HEIGHT OF THE CENTRE OF GRAVITY IN MAN, WITH SOME APPLICATIONS.

by

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The problem of finding the position of the centre of gravity of the body was first attempted by Borelli in 1679. An account of the various methods since employed has been published in 1922 by Croskey, Dawson, and others, who, moreover, attacked the problem on fresh lines. The historical account of the subject as given by these workers may briefly be summarized as follows:

In 1679 Borelli (1) attempted to determine the position of the centre of gravity in man by placing a subject supine upon a plank, which he pushed to and fro over a sharp supporting edge until equilibrium was attained. He then concluded that the centre of gravity of the subject lay in a vertical plane above the fulcrum. He failed to see the importance of balancing the plank apart from the subject.

The Weber brothers in 1836 improved the method by balancing the plank separately, and then, by pushing the subject to and fro obtained the equilibrium point. They found the centre of gravity to be 56·7 per cent. of the distance from the soles of the feet to the crown of the head. Meyer in 1853, employing Borelli's method, estimated the position of the centre of gravity as 59·5 per cent. of the distance from the soles to the crown. In 1857 Harless made a series of observations, giving as a mean result a point 57·99 per cent. of the length of the subject from the soles.

In 1890 Braune and Fischer adopted a fresh method. They froze a cadaver in the supine position, and transfixed it with three spits, each at right angles to the other two. The centre of gravity was then found by the orthodox method of suspending the body by each spit in turn and finding the point of intersection of the three vertical planes so determined. They completed but one experiment in full, giving the height of the centre of gravity as 54·8 per cent. of the distance from soles to crown.

Several investigators carried out experiments on this subject between the years 1918 and 1921 at the University of Wisconsin, and in 1922 Croskey,

Dawson, and others published an improved method. Their chief improvement was to screw the fulcrum to the plank so that it could be balanced once and for all. Further, they ruled parallel lines on the surface of the plank, so as to simplify the measurement of the distances of both soles and crown from the fulcrum. The balancing point was found as before by pushing the subject to and fro on the plank, and thus obtaining the equilibrium point by trial. In all, some 50 men and 50 women were measured. The mean figure in the case of the men was 56·18 per cent. of the total length from the soles, and in the case of the women 55·44 per cent. The subjects in all cases were measured fully clothed without shoes. In addition, a special investigation made on a number of women, unclothed, showed that the effect of clothing was practically negligible, lowering the centre of gravity about 0·2 per cent.

In 1923 the present writer developed two additional methods for determining the height of the centre of gravity. The first method may be referred to as the Direct Method, to distinguish it from the second or Differential Method, which is much the more accurate. The differential method has since been employed in this laboratory from time to time, to study changes in the centre of gravity of the human subject as affected by varying conditions of rest, respiration, posture, and exercise.

Having overcome certain difficulties, including the change of body length of the subject after exercise, and made a successful application of the differential method to these problems, it was decided to commence publication of a series of these studies. After this first paper on method and some applications was completed it was found that Palmer (1928), in his work describing a fresh method for determining the position of the centre of gravity in the antero-posterior plane, referred to a method of Du Bois Reymond for finding the height of the centre of gravity. This method appears to be essentially the same as the Direct Method here described, so that it cannot now be submitted as something new.

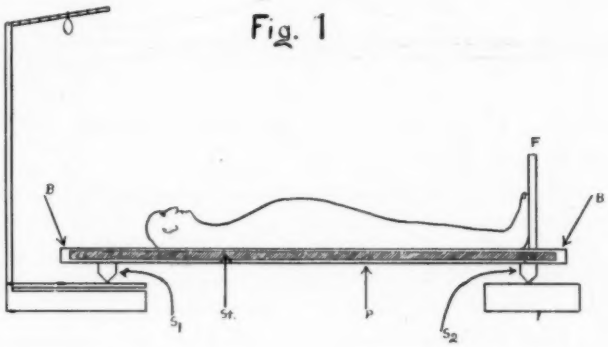
No description of the method is available, however, to authors previously quoted, to Palmer or to the present writer. The account is therefore left here as originally written, so as to lead up to a clearer explanation of the Differential Method.

Method 1 (Direct Method). This may best be described in terms of figure 1. In this figure P represents a strong flat plank approximately 2·4 metres long, 50 cm. broad, and 2·5 cm. thick. Along each side is screwed a strip of wood (B), 7 cm. high and 2·5 cm. thick. To this in turn is screwed a strip of steel (St.), 6 cm. high and 3 mm. wide. This arrangement prevents the plank yielding to any appreciable extent when supporting even a heavy subject. Near the ends are rigidly attached two supports, S_1 and S_2, which consist of wooden bars running at right angles to the length of the plank, and "shod" with angle

iron to form an approximation to the knife edges of a balance. The distance between the "knife edges" is exactly 2 metres.

A foot rest, F, is set up near one end, so that the surface which is to touch the soles of the subject is in the same vertical plane as the underlying "knife edge." This end of the plank may therefore be spoken of as the "caudal end," the other as the "cranial end." The upper surface of the wooden bar, B, on one side carries two metre rules, the zero end of one commencing at a point vertically above the "caudal knife edge," while the 100 cm. end of the second rule lies immediately above the "cranial knife edge."

Fig. 1



When the length of the subject is to be measured his head will be opposite some point on the second metre rule. Since his soles touch the foot rest, his height is given by adding 100 cm. to the reading shown on this rule.

The plank is placed so that the "cranial end" rests on the platform of a weighing machine and the "caudal end" on a smooth surface at the same level.

The weights throughout were given in pounds and ounces, and are so recorded here. No conversion to the metric system is made, as this quantity is eliminated in subsequent calculations.

The procedure adopted is as follows:

1. The weight of the subject is determined.

2. The plank *alone* is placed in the position shown in figure 1, and the reading on the machine recorded. Let this be w_1.

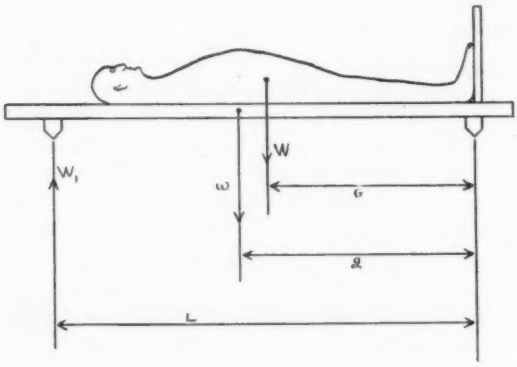
3. The subject then reclines on the plank, so that both soles make firm contact with the foot rest. Both arms are placed in a comfortable position parallel to the sides of the body, with fists closed but not clenched. This convention was adopted in preference to extended fingers, with a view to the

comfort of a subject who might be required to lie still for a period of half an hour or more in future experiments.[1]

The reading on the machine is now taken. Let this be W_1.

The conditions for equilibrium about the fulcrum of the "caudal knife edge" is shown by figure 2.

Fig. 2



Let L be the distance between the knife edges.

Let g be the distance of the Centre of Gravity of the plank system from the fulcrum.

Let G be the distance of the Centre of Gravity of the body from the fulcrum.

Then equating moments

$$W_1L = wg + WG$$
$$WG = W_1L - wg$$

but by previous measurement, w_1 acting at a distance L supports the plank system alone.

[1] The determination of the height (or length) of the subject is a matter for special consideration. This was done as follows:

1. The subject lies down on the plank, with stockinged soles touching the foot rest and with knees just slightly bent.

2. He next straightens out, and pressing firmly against the foot rest, pushes himself slightly along the plank into the standard position.

3. The head is next adjusted so that the gaze is directed at a mark on the ceiling previously determined to be vertically above his eyes.

4. A sliding device, carrying a vertical piece of wood, and adjusted so as to move along the plank and at right angles to it, is now moved to make firm but not hard contact with the subject's head.

As far as possible uniform pressure is applied so as to avoid a variable degree of compression of the hair.

5. The height is then measured on the second metre rule as already described which gives the excess over 100 cm.

It was considered advisable in the first instance to use a device for standardizing pressure against the head, but this was dispensed with in view of the accuracy of the results obtained.

Therefore $wg = w_1L$

so that $WG = W_1L - w_1L$

$$G = \frac{L\,(W_1 - w_1)}{W}$$

To illustrate by an example. Subject H.R. was measured.

Body length = 169·8 cm.

Weight = 143·45 lbs. = W

w_1 found to be 30·25 lbs.

W_1 found to be 98·85 lbs.

L = 200 cm.

$$\therefore\ G = \frac{200\,(98{\cdot}85 - 30{\cdot}25)}{143{\cdot}45}$$

$$= 95{\cdot}66$$

∴ the height of the Centre of Gravity expressed as a percentage of total height

$$= \frac{95{\cdot}66 \times 100}{169{\cdot}4}$$

$$= 56{\cdot}33$$

In practice it is found that some variation in the reading of the weighing machine is caused by breathing. In order to standardize the results, the subject is required to hold the breath at the end of a normal expiration.[2] This gives sufficient time to make the necessary reading.

In order to test the accuracy of the method a number of measurements were then made upon the same subject. In Table 1 are recorded a series of ten consecutive measurements on H.R. In order to make each measurement quite an independent one, the subject was required to get off the plank and stand up each time a measurement was made.

TABLE 1.

Determination of the Height of the Centre of Gravity in the case of H.R. (Weight 143·45 lbs.) by the Direct Method.

	Height in cm.	$W_1 - w_1$ in lbs.	Height of C. of G.	C.G. height as per cent. of body length.
	169·40	68·3	95·35	56·25
	169·55	68·5	95·60	56·40
	169·80	68·7	95·90	56·45
	169·50	68·3	95·35	56·25
	169·80	68·6	95·70	56·35
	169·80	68·6	95·80	56·40
	169·85	68·5	95·60	56·30
	169·55	68·5	95·65	56·40
	169·40	68·1	95·10	56·15
	169·55	68·4	95·45	56·30
Mean	169·62		95·55	56·32

[2] Expiration is chosen rather than inspiration, as the subject, knowing that he is about to hold the breath, is less likely to exceed his normal respiratory movement at this phase than at the phase of normal inspiration.

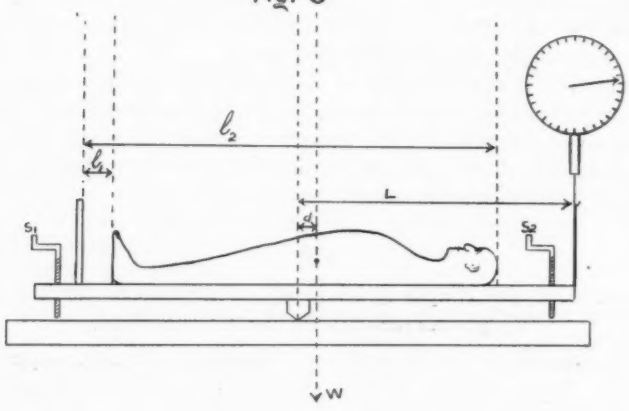
The per cent. values show a standard deviation of 0·087, while the coefficient of variability (i.e., $\frac{\text{S.D.} \times 100}{\text{Mean}}$) = 0·154. The P.E. of the mean is 0·020.

Method 2 (The Differential Method). The essence of this method is to place the subject upon a plank (already balanced about a steel edge), so that the system (plank + subject) tends to fall at the cranial end, and to measure the force applied at the end of the plank to maintain equilibrium. This force is best measured by means of a spring balance, to save adjusting weights and to give immediate results.

In practice a circular Salter's balance was used, graduated in pounds and ounces. The same plank was used as in the previous method, an additional support provided with a "knife edge" being fixed to the middle of the board, which may then be balanced.

The calculation of the distance of the centre of gravity from the fulcrum is illustrated by figure 3.

Fig. 3



Let w be the supporting force maintaining equilibrium (reading of the spring balance).
Let W be the weight of the subject.
Let L be the distance between the fulcrum and the end of the plank.
Let d be the distance between the fulcrum and the Centre of Gravity of the body.
Then $w.L = W.d$
$d = w.L/W$

Now l_1 and l_2 are measured by means of the sliding piece previously mentioned, so that the total height $= l_2 - l_1$
and as the height of the Centre of Gravity $= 100 - l_1 + d$

$$\text{the height of the C. of G. as percentage of total height} = \frac{100 + d - l_1}{H} + 100$$

$$= \frac{100\,(100 - l_1 + \frac{w.L}{W})}{l_2 - l_1}$$

As in the case of the previous method, the supporting weight varies with the phases of respiration, so that the readings are taken with the subject holding the breath at the phase of normal expiration.[3]

To convert the plank system or balance table from a freely swinging system to a fixture, and *vice versa,* a screw bolt is affixed near each end (shown in figure 3 as S_1 and S_2). When the attached handles are given a few turns the bolts descend, and the table is fixed and ready for the subject. A few turns in the opposite direction elevate the bolts and leave the table free to swing and ready for use.

An example will serve to illustrate the method.

In the case of J.R., W was 135·5 pounds

l_1 was 10·5 cm.

l_2 was 185·1 cm.

w was 10·9 pounds

L was 120 cm.

Whence the percentage height of the Centre of Gravity

$$= \frac{100\,(100 - 10{\cdot}5 + \frac{10{\cdot}9 \times 120}{135{\cdot}5})}{185{\cdot}1 - 10{\cdot}5}$$

$$= 56{\cdot}8$$

This method of measuring the length of the subject was found to be quite unsatisfactory. It involves one of the defects of all previous methods for finding the centre of gravity, that there is no fixed surface to keep the soles vertical. It is easy to make an error of 1 cm. in making this measurement.

A further modification of the Differential Method was then adopted to obviate this difficulty. In this method the height is measured as in Method 1. Small subjects, however, in this position have a value of h less than 100 cm., so that the balance table tends to tilt caudally. To overcome this, two horizontal steel rods, each about 90 cm. long, were attached to one side of the apparatus, each carrying a rather heavy sliding weight of cylindrical shape.

The procedure then runs:

1. The table is balanced by adjusting the sliding weights, and then fixed by means of the screw bolts S_1 and S_2.

2. The subject reclines upon it as directed.

[3] The effect of respiration on the supporting weight is brought about in the following manner. When inspiration occurs and the diaphragm descends, the viscera are pushed in a caudal direction, so that the Centre of Gravity must also move caudally, thus lessening the supporting force w.

3. The height is measured as in Method 1.

4. The screw bolts are turned to allow the table to swing free.

5. (a) If the table now tilts cranially a reading is made on the spring balance, and d is calculated. (b) If the table tends to tilt caudally, the sliding weights are adjusted in any position to tilt cranially, and the reading taken as before. Call this reading w.

The table is again "fixed," the subject gets off, and the reading of the scale again taken, which, of course, must be greater than w. Call this Z. The balancing force, as far as the body alone is concerned, must therefore be Z — w *acting downwards.*

$$\text{So that } (Z - w)L = W.d.$$

$$\text{From which } \quad d = \frac{(Z - w)L}{W}$$

In this case $h = 100 - d$, which simplifies the calculation.

$$\text{The complete expression is now } 100\left(\frac{100 - \frac{(Z - w)L}{W}}{H}\right)$$

Accuracy of Method 2. The accuracy of the method was then tested by a series of measurements on the same subject as in Method 1. These are given in Table 2.

TABLE 2.

Determination of the Height of the Centre of Gravity in the case of H.R. (Weight 143·45 lbs.) by the Differential Method.

	Length of Subject.	Balancing Force in Pounds *Downwards.*	Distance from Fulcrum to C. of G. in cm.	Height of C. of G. in cm.	Height of C. of G. as per cent. of length.
	169·35	6·4	—5·40	94·60	55·85
	169·50	6·2	—5·20	94·80	55·95
	169·20	6·5	—5·45	94·55	55·90
	169·35	6·25	—5·25	94·75	55·95
	169·45	6·2	—5·20	94·80	55·95
	169·40	6·25	—5·25	94·75	55·95
	169·45	6·25	—5·25	94·75	55·90
	169·40	6·25	—5·25	94·75	55·95
	169·50	6·3	—5·30	94·70	55·85
	169·35	6·2	—5·20	94·80	55·95
Mean	169·39			94·72	55·93

In this case the S.D. of the per cent. values obtained is 0·041, and the coefficient of variability 0·073. The P.E. of the mean is 0·009.

On comparing Tables 1 and 2 it will be seen that the means differ by a very significant amount, in view of the agreement of individual determinations in each series. The question arises as to whether the difference is due to some

constant error coming into play in one of the methods, or whether the centre of gravity of the subject was really different on the two occasions.

To test the matter both methods were employed to locate the centre of gravity of a heavy piece of hardwood weighing about 57·5 lbs. Five measurements were made by each method in different positions of the object. The results are given in Table 3.

TABLE 3.

A Series of Determinations of the Position of the Centre of Gravity of a Heavy Piece of Hardwood by Methods 1 and 2.

Sequence of Determinations.	Distance of C. of G. from End. Method I.	Method II.
1	78·51	78·64
2	78·75	78·55
3	78·79	78·75
4	78·62	78·72
5	78·72	78·68
Means	78·68	78·67

The *very* close agreement between the means is seen to be merely a matter of chance, since the constituent values in each case have a much greater variation. A study of the individual values, however, shows that the standard deviations of the two series are 0·10 and 0·05 respectively. Hence we may safely conclude that no significant error has crept into the methods employed, and that the difference in the centre of gravity of H.R. at different times represents a real change. The reality of this difference may further be checked by statistical methods from the mean of each series and the P.E. of each mean $(P.E.)_1$ and $(P.E.)_2$ respectively, making use of the method cited by Dunn.

Thus the difference between the two means $= 56·32 - 55·93 = 0·39$ (1)

The P.E. of this difference

$$= \sqrt{(P.E.)_1^2 + (P.E.)_2^2}$$

$$= \sqrt{(0·020)^2 + (0·009)^2}$$

$$= 0·022 \quad .. \quad (2)$$

From 1 and 2, according to the probability tables, the odds against such a difference in the two series occurring by chance runs into many millions to 1.

It was evident in preliminary experiments with the differential method that its accuracy was limited, not only by the coarseness of the graduation (ounces), but also by the difficulty of reading the spring balance. This was due to the oscillations of the balance index caused by the heart-beats.

With a view to testing the limits of accuracy of the method, a writing point was attached to the index of the spring balance, and its movements recorded on the surface of a moving drum. When the subject holds the breath, the usual respiratory excursions of the index cease, and smaller oscillations, due to the heart-beats, are recorded. A horizontal line drawn through half the height of these gives the mean position of the index.

By suitable calibration it was then found possible greatly to refine the accuracy of measuring the force exerted by the spring balance. A series of such records was taken while the data were being obtained for the compilation of Table 2. These results are set out in Table 4, showing a very considerable increase in accuracy.

TABLE 4.

Refinement of the Differential Method by a Graphic Record of the Mean Position of the Scale Index to Measure the Balancing Force.

Subject H.R., Weight 143·45 lbs.

	Length of Subject.	Balancing Force (downward).	Distance from Fulcrum to C. of G. in cm.	Height of C. of G. in cm.	Height of C. of G. as per cent. of length.
	169·35	6·36	—5·34	94·66	55·89
	169·50	6·19	—5·20	94·80	55·94
	169·20	6·49	—5·45	94·55	55·89
	169·35	6·23	—5·23	94·77	55·95
	169·45	6·20	—5·21	94·79	55·93
	169·40	6·26	—5·26	94·74	55·92
	169·45	6·25	—5·25	94·75	55·91
	169·40	6·26	—5·26	94·74	55·92
	169·50	6·31	—5·30	94·70	55·88
	169·35	6·20	—5·21	94·79	55·96
Mean	169·39			94·73	55·92

In this case the results show a closer agreement than before, the S.D. being 0·026 and the coefficient of variation 0·014. The P.E. of the mean is 0·006.

It may seem at first sight over precise to attempt the degree of accuracy quoted. The centre of gravity, however, varies so little in different subjects, and in the same subject from time to time, that no meaning may be ascribed to differences unless they can be determined with an exactitude of a very high order. This will be more evident in the discussion of the application of these methods in the last section of this paper.

Discussion of Errors. The following sources of error were investigated:

1. The error due to variations in the precise position of the head. A series of measurements was made on two subjects, in which the head alone was moved each time and then brought back to the standard position. The S.D. of the series of variations so found was small in each case, being 0·09 cm. and 0·06 cm. respectively.

2. The error due to variable compression of the hair. Two sets of measurements gave S.Ds. of 0·07 cm. and 0·05 cm. respectively.

3. The error due to inability of the subject to assume precisely the same posture on each occasion. This error cannot be measured separately, but all three errors, together with variations in the spinal curves, affect the determination of height.

Some subjects exhibit much greater variations than others, doubtless due to differences in their skill in assuming a constant posture. Practice diminishes this error, e.g., one subject in a series of ten measurements showed a maximum height of 178·2 cm. and a minimum of 177·3 cm., with a S.D. of 0·26 cm. On the other hand the chief subject of this communication had a moderate amount of practice. In an earlier series his S.D. was 0·19 cm. In a later series his S.D. was 0·08 cm.

It was observed, however, that a variation in the height tends to be compensated for, to some degree, by a corresponding change in the height of the centre of gravity, so that the final result is not disturbed so much as might be expected.

This parallel change is reflected in the calculation of the coefficient of correlation between the height of the centre of gravity and the total height. In two series where this coefficient was calculated it was $0{\cdot}66 \pm 0{\cdot}12$ and $0{\cdot}59 \pm 0{\cdot}14$ respectively.

4. The error in determining the balancing weights. In the Direct Method this error would in general produce an error less than 0·2 cm. in the height of the centre of gravity, in the Differential Method an error well below 0·1 cm.

5. A slight error may be introduced if the balance board be not quite level. This is very small, and may in general be safely neglected. A correction for this error was applied at first, but later obviated by using a small windlass attached to the end of the balance table to vary the length of the cord connecting this with the spring balance. By this means, when equilibrium is established, the table may be made horizontal, as shown by an attached spirit level, in a few seconds. The small windlass is provided with a 45° worm wheel, which winds quickly and yet with sufficient friction to remain fixed in any position with the small balancing forces employed.

Applications. The differential method was employed to measure the height of the centre of gravity in 45 men and 38 women mostly between the ages of 19 and 22 years. In all cases the subjects were measured fully clothed, but without shoes. The results are set out in Table 5.

TABLE 5.

Height of the Centre of Gravity in 45 Men and 38 Women. Numbers mean per cent. of sole–crown length (excepting the coefficient of variation).

	Men.	Women.
Mean	56·74	56·07
Minimum	55·2	54·7
Maximum	57·9	57·3
Standard Deviation	0·65	0·67
Median	56·67	56·17
Lower Quartile	56·36	55·56
Upper Quartile	57·34	56·63
Probable Error	0·49	0·53
Coefficient of Variability	1·15	1·69

As no previous work mentions the effect of clothing in men, it was thought worth while to assess this factor. This was done in the case of six of the men. The obvious method of measuring the centre of gravity of the men clothed and then without clothing was not chosen, since it was thought possible that the differences obtained might be due largely to the inability of the novice to take precisely the same position on the table on different occasions.

It was therefore decided to measure the men fully clothed, without shoes, and then find the centre of gravity of the clothing separately when arranged on the table corresponding to their position on the subject. The results show that clothing produced a difference in the centre of gravity of the men no greater than might be obtained in two successive measurements on the same clothed subject. The same type of experiment was then repeated for comparison in the case of five of the women.

The essential data and results are set out in Table 6.

TABLE 6.

Effect of Clothing in Altering the Centre of Gravity of the Body.

Subject.	(1) Height in cm.	(2) Height of C. of G. in cm.	(3) Height of C. of G. of Clothes in cm.	(4) Ratio of Body Weight to Weight of Clothes (to nearest 0·5).	(5) Column (3) minus Column (2).	(6) Deflection of C. of G. of Body as per cent. of Height.
Men.						
E.B.	180·7	101·9	102·6	25·5	+0·7	+0·02
L.P.	178·9	100·1	102·6	21·5	+2·5	+0·05
R.D.	173·1	98·2	101·3	24·5	+3·1	+0·07
I.B.	171·4	97·1	100·0	27·0	+2·9	+0·06
A.L.	176·9	102·3	102·2	24·0	—0·1	0·00
H.A.	174·7	98·3	101·7	22·0	+3·4	+0·08
Mean						+0·05
Women.						
N.H.	167·2	93·2	89·3	62·0	—3·9	—0·04
D.M.	168·0	96·4	93·3	47·0	—3·1	—0·05
H.B.	176·5	99·3	98·0	49·5	—1·3	—0·01
E.F.	154·7	88·0	91·3	56·0	+3·3	+0·04
E.B.	165·6	94·0	92·8	48·5	—1·2	—0·03
Mean						—0·02

Although the number of experiments is small, it is sufficient to indicate the order of magnitude of the deflection of the centre of gravity due to clothing. In none of the eleven cases does it amount to 0·1 per cent. of the total height, so that it represents at most a very minor error.

The results for men and women are, however, reasonably consistent within themselves, indicating a slight tendency for the clothing to elevate the centre of gravity in men and to lower it in the case of women. This tendency to widen the difference in the mean values for men and women is only of the order of

about 10 per cent. of the actual difference. The sex difference as determined by these experiments is therefore real, and not due to the differences in clothing.

The Differential Method, modified by taking a tracing from the pointer of the spring balance, may be employed to obtain some index of the abdominal component of respiration. By measuring the amplitude of the respiratory variations it is possible to express this change as equal to a definite variation in the supporting force. This may in turn be expressed in two ways:

1. As an oscillation of the centre of gravity of the body to the extent of so many millimetres or so much per cent. of the total height.
2. As the equivalent of moving a certain mass a definite distance on the balance table.

An example may be given to illustrate this.

In the case of M.H., at rest on the balance table, the mean respiratory variation is equal to 65·8 grm. applied at the end of the table, namely 120 cm. from the fulcrum.

Since the weight of the subject was 64·0 kilos, and the height 177·6 cm., this variation is equivalent to a range of movement of the centre of gravity of 1·24 mm., or 0·067 per cent. of the height. Alternatively it may be expressed as a to and fro displacement of mass equal to $120 \times 65{\cdot}8$ grm. cm. = 7896 grm. cm. approx. = 7·9 kilogram cm. approx.

A further application may be made to measure the change of the centre of gravity of the body as a result of vigorous exercise, such as running, due to the increased amount of blood in the muscles of the lower limbs. This may be compared with the changes produced by standing still for five minutes and by standing and walking about for a much longer period during ordinary laboratory activity.

To illustrate by one[4] example. The subject (A.M.A.), whose weight was 61 kilos., and height 171 cm., rested after ordinary laboratory activity for about 30 minutes, during which period a tracing was taken by means of the balance pointer, which was caused to write upon a moving surface. In this period a constant level was reached. He then stood still for 5 minutes, when a further tracing was taken for some fifteen minutes more, until constancy of level was established. He then ran twice up and down a flight of steps 36 feet high in 1 minute and 20 seconds. At the end of the run he climbed on to the balance table with a minimum loss of time and took up the standard posture. A tracing was again taken, reaching constant level in about 30 minutes.

By calibrating the record, the supporting force w was calculated at the beginning and at the end of the tracing. The results then calculated are set out in Table 7.

[4] This example was chosen as the data were not complicated by any significant change in the length of the subject during the experiments.

TABLE 7.

Showing the Displacement of the Centre of Gravity under Varying Conditions of Rest and Exercise.

(By the observation period is meant the period during which the tracing was taken.)

Activity or Rest prior to Observation Period.	Difference in balance Readings converted to gm. (beginning and end of observation period).	Difference produced in C. of G. in mm.	Equivalent Displacement of Mass in kilogram cm.
Stood or walked around laboratory for about half-an-hour	111	2·21	13·3
Stood still for 5 mins. after 30 mins. supine	36	0·72	4·3
Ran twice up and down a flight of steps (36 feet) in 80 seconds ..	271	5.38	32·5

The data show that as a result of the exercise plus change of posture there occurred a displacement of mass, presumably blood, to the extent of 32·5 kilogram cm. in the caudal direction. A study of these changes in athletic and non-athletic subjects is now proceeding.

SUMMARY.

1. A brief review is given of the methods hitherto employed in measuring the height of the centre of gravity in man.

2. A new method is described, which is claimed to be an improvement upon existing methods, alike in respect to accuracy and rapidity.

3. This method, which is essentially a differential method, is shown to give the same results within the limit of experimental error as a method attributed by Palmer to Du Bois Reymond, by applying them both to find the centre of gravity of a fairly heavy beam.

4. The various sources of error in these two methods are compared and evaluated. All these are small. In the differential method errors other than the measurement of the height of the subject are by comparison quite negligible.

5. Applications.

(a) The height of the centre of gravity was measured in the case of 45 men and 38 women. The values obtained were in both cases a little higher (about 0·6 per cent.) than those obtained by Croskey, Dawson, and their co-workers, leaving the sexual difference almost identical. The difference due to clothing was shown to be practically negligible in both sexes.

(b) This new method is sufficiently accurate to show that the centre of gravity of the same subject exhibits distinct variations from time to time apart from the respiratory variation.

(c) It was found possible to determine the extent of fluctuation of the centre of gravity during the respiratory cycle. This variation may also be expressed as an index of the extent of the abdominal component in respiration.

(d) After a period of standing or walking about the laboratory the centre of gravity falls, but on lying quietly on the balance table for some 20 to 30 minutes it gradually rises to a constant level. This represents, not merely an absolute rise of the centre of gravity such as might be due to an increase in total height of the subject, but a relative increase in per cent. of the total height.

(e) This caudal movement of the centre of gravity is accentuated during muscular exercise. The change may also be expressed as the equivalent of a mass displacement of so many kilogram cm. In the case cited it was 32·5 kilogram cm. This is interpreted to mean a migration of blood to the lower limbs during exercise, with a return after a period of recumbency.

In conclusion I wish to express my thanks to Professors H. G. Chapman and H. W. Davies, in whose laboratory the work was carried out, to Professor H. Priestley for helpful criticism, to Miss E. M. Hindmarsh for assistance with some of the measurements, and to those serving as subjects for the research.

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THE STANDARD METABOLISM OF AUSTRALIAN ABORIGINALS

by

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The present communication is practically an interim report, the publication of which in its manifest incomplete state is the result of circumstances. It represents work undertaken for the Carnegie Institute of Boston at the request of Dr. Francis G. Benedict, as part of a general metabolic survey of aboriginal races throughout the world, and, falling within the sphere of action of the Australian National Research Council's Anthropological Committee, the expenses of the expedition were defrayed by funds received from the Rockefeller Foundation and administered by that body. Owing to the difficulties encountered in the field, it became evident to us that considerably more work was necessary before a satisfactory statistical analysis could be made. Added to this was the further difficulty imposed by discussions per medium of correspondence with Dr. Benedict. Finally, it was decided by Dr. Benedict that the results should be published, if only as an "interim report," leaving a final statement for such time as circumstances may permit another investigation to be made.

Method. The Benedict portable apparatus was used for determining the oxygen consumption, while the Douglas bag and Haldane gas analysis apparatus were taken for respiratory quotient investigation. Unfortunately a temperature fall of 35°F. in the course of two hours fractured the potash bulb of the latter, and this aspect of the research was discontinued. A Knipping portable alveolar CO_2 apparatus was used for estimating the closed alveolar CO_2 percentage as a check upon muscular activity or food ingestion prior to the measurements. The Benedict portable apparatus proved excellent in the field, the natives rapidly accustoming themselves to its use, after a demonstration indicating to them how to breathe into the mouthpiece. The chief difficulty lay in occluding the nostrils, which were not only large and widely spaced, but in many instances strengthened by extraordinarily firm cartilages. In these cases an assistant closed the airway by hand.

Dietary. All the females and children were fed in the Mission Hostel on the following: Breakfast, one cupful bread and milk, wheatmeal porridge, or sago; 2 pieces (loaf section) of bread and jam; tea with sugar but no milk. Dinner, stew, consisting of meat, potato, and onion, largely vegetable, 1½ oz. each person; bread and jam (2 pieces); tea as above. Afternoon tea, 1 piece of bread or a handful of biscuits; no tea. Tea (evening meal), bread and butter, 4 or 5 pieces per person; tea as before; occasional biscuits.

The natives are all pure-blooded aboriginals of the Kokata tribe, collected at the Koonibba Mission of the Evangelische Lutheranische Kirche of South Australia, and to Pastor Hoff and Mr. Braunach, of that mission, we owe much for their patience in persuading natives to submit for experimentation. Owing to three years of drought, many natives had camped about the Mission Station, in order to draw Government rations, and this explains the number available for investigation, a number not readily collected together in ordinary times.

Experimentation began daily at 5 a.m., the subjects being in the so-called post-absorptive state, and the appended table, which, apart from some corrections to the field notes, was compiled by the Carnegie Institute staff, summarizes the investigations.

DISCUSSION.

It appears at once from the data, that although the young children are included, largely because they were under the direct control of the mission, and gave us good starting material, from the standpoint of racial metabolism they should be excluded, owing to the difficulties of prediction. These difficulties are, in our opinion, peculiarly great for people of an entirely different configuration from Western types, as these unquestionably are. Harris and Benedict do not extend their tables below 21 years. Aub and DuBois include ages as low as 14 years, stating that the metabolism of the female is 7 per cent. lower than that of the male in this age group. The question of configuration, however, already referred to, is one of greater difficulty for all groups, if a comparison with Western standards is to be instituted. The von Pirquet index, or pelidisi, would seem to indicate a better state of nutrition than that which actually exists among these aboriginals.[1] The general average for Western

[1] According to von Pirquet the cube of the sitting height expressed in centimetres approximately equals the weight in grams of a normal subject. The ratio termed the pelidisi is obtained as follows:

$$\frac{W \times 10}{(S.H.)^3} = 100\%$$

or $$\frac{\sqrt[3]{W \times 10}}{S.H.} = 100\%$$

Where W = Weight in grams,
S.H. = Height (sitting) in centimeters (2).

human beings is estimated by Harris and Benedict as 94 for men and 96 for women. According to Benedict the general average or ideal pelidisi assumed by von Pirquet was 98; while his own experience at the Nutrition Laboratory rarely included figures as high as 108 (1). Among the present subjects there are 23 whose von Pirquet index is 100 or more, and of these 6 are over 107. These figures have been checked where possible by comparison with anthropological measurements made by a separate expedition from the University of Adelaide among these natives. The fact is that although the diet of these natives is minimal, their physical condition is not so poor as might be anticipated. From conversations with other field investigators it appears that when subsisting in a true state of nature, upon roots, large grubs, snakes, etc., the native appears to be in better condition than when living on rations from a sheep station or mission, despite the regular receipt of the latter. Among the natives studied the following were in what might be termed good physical condition:

Expt. No.	Pelidisi.	Expt. No.	Pelidisi.	Expt. No.	Pelidisi.
4	108	25	110	43	113
5	102	26	108	47	109
6	102	28	105	54	108
21	106	31	113	55	113
20	103				

On the other hand, there are almost as many cases of high pelidisi, in which the subject could not be described as being in good condition. It is apparent then that we are dealing with a special bodily configuration, and that the pelidisi is not an accurate index of nutrition so far as these subjects are concerned. It might be added that the term "good condition" does not imply any degree of adiposity, for among the natives adiposity is rare, and in the present case the term could be applied only to Nos. 31 and 47.

The diet of those natives living in camp in the vicinity depended almost entirely upon the mission ration of flour and sugar, which could not be considered a "stimulating" diet, even in those few cases where measurements were made within the usual "post-absorptive" period. In accordance, however, with a conservative view, such cases have not been computed (cf. table).

Unfortunately, we had not katathermometer, and wet and dry bulb thermometer readings were the sole guide to weather conditions. Koonibba lies within the 11 inch rainfall belt, and therefore has an arid climate. The thermometer was always placed in such a position as to record variations as they affected the subject. So far as the metabolic summary is concerned, the last column of the table gives Aub and DuBois predictions, which are believed by Benedict to be 5% too high for American women. For adult women the Aub and DuBois and the Harris and Benedict predictions are essentially the same. This being so, lower figures for aboriginal women than those predicted might be anticipated. Although in the case of L.E., experiment 15 gives a much lower

figure than the two preceding experiments, it may be included, as this was the most favourable of a difficult series.

Thus we have for the women:

Name.	Deviation.	Name.	Deviation.	Name.	Deviation.
B.P.	+10	E.H.	—5	B.J.	—20
A.K.	—12	E.M.	—27	Lydia	—21
F.N.	—8	G.B.	—36	Irene	—14
E.P.	—23				

Judged by Caucasian standards the average is strikingly low, being approximately —16%. Allowing for the 5% correction above mentioned, we still have a figure of —11% for these women. The climate, being arid, does not compare in humidity with that of the Sydney experiments (3). It was common experience that as we continued the experimentation on a given native the oxygen consumption fell, i.e., the subject became more used to experimentation. It may be assumed without reading more into the figures than is superficially obvious, that there is definitely lower metabolism *measured by European standards.*

In the case of the males, leaving out cases under 12 years, and those where food was taken, there is an average for some 17 subjects of —8%.

Name.	Deviation.	Name.	Deviation.	Name.	Deviation.
C.H.	—9	W.E.	—22·3	E.P.	—20
A.P.	—13	P.K.	—8	H.Y.	—14·6
E.P.	—13	F.G.	—18	L.W.	—3·5
T.M.	—6	C.H.	—25	L.M.	—14
J.N.	—11·4	F.C.	—15	C.K.	—11
G.G.	—14	W.W.	—17		

K.B. was an aberrant case with high pulse; we considered him to be suffering abdominal complaint. Although the figures vary from case to case, and with the same case from time to time, having regard to the circumstances of the experiments, it can be stated that again a definitely low metabolic picture is presented.

To the extent that the pelidisi of these natives point to good nutrition, the results are the more striking; it is doubtful, however, if this index is applicable without modification, but one thing is certain, and it is in keeping with the picture of low metabolism, namely, these natives do unquestionably appear better nourished on a meagre diet than white subjects would be. The mission ration is appended, and the natives had little opportunity of increasing the diet, as they could get little or no work to earn money. They hunted for snakes and rabbits, but owing to drought these were scarce, and when caught were divided among a large number, so that each individual received only a small share. In any case, the dietary at the Mission Station itself was all that the females received, and they appeared to be in the same condition as those outside. They are a curious people, giving one the impression that they are superlatively lazy and inactive, but the fact remains that the men can and do form

most successful football teams, in which their success is due entirely to their running speed, in which, in the case of the mission team, formed from natives casually employed at farm labour, they surpass their white opponents.

SUMMARY.

The gaseous metabolism of some 40 Australian aboriginals of the Kokata tribe has been estimated by the Benedict field portable apparatus, 140 separate measurements being made.

The oxygen consumption indicates a deviation of at least —11% for females and —13% for males, using European standards of measurement. Deviations as high as —36% were encountered.

Measurements of standing and sitting height led to the discovery of a von Pirquet index of over 100 in 52% of subjects studied, 16% having an index greater than 108.

In view of these abnormal pelidisi measurements it is considered doubtful as to whether European standards for computing basal metabolism are applicable in these cases.

Our thanks are due to Dr. Francis G. Benedict for his very patient assistance and advice, and to the Carnegie Institute (Nutrition Laboratory), Boston, for the field apparatus used. The travelling and maintenance expenses were defrayed by a special grant from the Anthropological Committee of the Australian National Research Council from funds received from the Rockefeller Foundation.

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Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height *and* $SH-\frac{H}{2}$	(SH) Sitting Height *and* *Pelidisi*	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Harris, Benedict, or Girl Scout.	Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.cent.	sq.m.	cal.	cal.	p.cent.
L.B. ♂ 1	11	29·7	138 *+0·2*	69·0 *97·0*	66·0	Dec. 28, '28	37·4	60 60	22·0	—	—	12	182 182							
Avg.								60					182	1,265	1,091	+15·9				
L.B. ♂ 3	11	29·7	138 *+0·2*	69·0 *97·0*	66·0		36·1	52 52	18·0	22·8	17·8	12½	161 163							
Avg.								52					162	1,126	1,091	+3·2				
C.H. ♂ 2	15	44·7	152 *+1·5*	77·5 *98·0*	75·6	Dec. 28, '28	35·9	52 53	18·0	—	—	12	206 194							
Avg.								52					196 199	1,383	1,340	+3·2	1·38	1,002	1,104	—9·2
E.P. ♀ 4	23	51·0	153 *—2·8*	73·7 *108·0*	81·9	Dec. 30, '28	36·7	73 70	15·0	—	—	12	206 216							
Avg.								72					211	1,466	1,318	+11·2	1·47	997	888	+12·3
E.P. ♀ 8	23	51·0	153 *—2·8*	73·7 *108·0*	81·9	Jan. 1, '29	36·9	66 66	26·0	24·4	16·3	12¾	231 208							
Avg.								66 66					199 204	1,417	1,318	+7·5	1·47	964	888	+8·6
A.K. ♀ 5	19	50·8	154 *+1·1*	78·0 *102·0*	81·3		36·1	70 70	16·5	—	—	13	176 179							
								170					175		1,107†	+9·8†				
Avg.								70						1,216	1,337	—9·1				
															1,250*	—2·7*	1·47	827	912	—9·3
A.K. ♀ 9	19	50·8	154 *+1·1*	78·0 *102·0*	81·3	Jan. 1, '29	36·7	66 66	26·5	25·5	17·5	13½	167 170							
								66					155		1,107†	+2·9†				
Avg.								66					164	1,139	1,337	—14·8	1·47	775	912	—15·0
															1,250*	—8·9*				

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height and $SH-\frac{H}{2}$	(SH) Sitting Height and *Pelidisi*	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Heat produced per 24 hours. Harris, Benedict, or Girl Scout.	Heat produced per 24 hours. Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.ct.	sq.m.	cal.	cal.	p.ct.
L.E. ♀	21	50·3	152	77·5	81·3	Jan. 1, '29	37·2	84	27·0	26·8	20·0	14½	195							
10			*+1·5*	*102·0*				70					196							
								70					153							
Avg.								77					181	1,258	1,319	—4·6	1·45	868	888	—2·3
L.E. ♀	21	50·3	152	77·5	81·3	Dec. 30, '28	36·7	70	18·0	—	—	14½	182							
6			*+1·5*	*102·0*									189							
Avg.								70					186	1,292	1,319	—2·0	1·45	891	888	+0·3
L.E. ♀	21	50·3	152	77·5	81·3	Jan. 3, '29	37·0	84	25·5	26·1	18·9	12¾	157							
15			*+1·5*	*102·0*									190							
Avg.								84					157	1,091	1,319	—17·3	1·45	752	888	—15·3
F.N. ♀	14	46·1	154	74·9	78·7	Dec. 31, '28	36·7	70	19·0	21·1	16·1	13	195							
6			*—2·1*	*103*				68					192							
													188		1,205†	+10·7†				
Avg.								69					192	1,334	1,314	+1·5	1·41	946	1,032	—8·3
															1,250*	+6·7*				
I. ♀	14	39·0	154	73·7	73·7	Dec. 31, '28	37·0	74	23·0	23·9	16·7	13¾	168	1,167	1,022†	+14·2†				
7			*—3·3*	*99·0*											1,247	—6·4	1·31	891	1,032	—13·7
															1,250*	—6·6*				
I. ♀	14	38·6	154	73·7	73·7	**Jan. 1, '29**	35·6	84	28·5	28·3	18·9	1	171							
11			*—3·3*	*98·0*				84					164							
I. ♀	14	38·6	154	73·7	73·7	Jan. 2, '29	36·7	90	19·0	16·7	14·4	12½	154							
13			*—3·3*	*98·0*				**90**												
															1,022†	+13·9†				
Avg.								90					165	1,146	1,247	—5·2	1·31	875	1,032	—15·2
															1,250*	—5·3*				
E.P. ♀	14	42·6	153	74·3	74·9	Jan. 1, '29	37·0	90	—	26·5	22·2	4	225							
12			*—2·2*	*102·0*				88					198							
								89					179							
Avg.								89					201	—	—	—	—	—	—	—
E.P. ♀	14	42·6	153	74·3	74·9	Jan. 2, '29	36·7	84	20·0	17·9	15·6	13¼	194							
14			*—2·2*	*102·0*				84					158		1,250*	—12·2*				
													185		1,280	—14·2	1·36	807	1,032	—21·8
								84					158	1,098	1,116†	—1·6				
E.P. ♀	14	42·6	153	74·3	74·9	Jan. 3, '29	36·7	90	27·0	26·3	19·4	13½	186							
16			*—2·2*	*102·0*				87					176		1,250*	—15·0*				
								88					153		1,280	—17·0				
Avg.								88					153	1,063	1,116†	—4·7†	1·36	782	1,032	—24·2

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height *and* $SH-\frac{H}{2}$	(SH) Sitting Height *and* *Pelidisi*	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Harris, Benedict, or Girl Scout.	Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.ct.	sq.m.	cal.	cal.	p.ct.
D.N. ♂ 9	9	20·0	117	60·3	53·3	Jan. 3, '29	36·8	96	27·0	27·2	20·0	14½	134	931	866	+7·5				
17			*+1·8*	*98·0*																
A.P. ♂ 19	19	53·5	163	76·3	66·7	Jan. 6, '29	36·2	54	17·5	16·7	14·0	12½	195							
21			*—1·8*	*106·4*				54					189							
Avg.								54					192	1,334	1,359	—1·8	1·56	855	984	—13·1
E.P. ♂ 20	20	60·3	166	81·5	82·6	Jan. 5, '29	36·2	54	18·5	18·3	15·6	13¾	207							
20			*+1·3*	*103·6*				54					175							
Avg.								54					191	1,327	1,466	—9·5	1·70	780	948	—13·0
T.M. ♂ 28-30	28-30	76·0	172	84·0	92·7	Jan. 7, '29	36·4	78	19·5	20·0	16·7	12¼	250							
22			*+1·8*	*108·4*				70					254							
								—					250							
Avg.								74					251	1,744	1,641	+6·3	1·88	927	948	—4·7
T.M. ♂ 28-30	28-30	76·0	145	74·3	92·7	Jan. 8, '29	36·0	54	18·0	17·8	15·5	12¼	238	1,654	1,641	+0·8	1·88	880	948	—7·1
23			*+1·8*	*123·0*				54												
J.N. ♂ 26	26	72·9	173·5	85·7	88·9	Jan. 8, '29	36·3	60	19·5	18·3	16·1	13	240							
24			*+1·0*	*105·0*				60					221							
													236							
Avg.								60					232	1,612	1,744	—7·6	1·87	862	948	—9·1
J.N. ♂ 26	26	72·9	170	85·7	88·9	Jan. 9, '29	36·2	60	17·0	16·1	15·0	13	224							
27			*+0·7*	*105·0*				60					220							
													220							
Avg.								60					221	1,536	1,744	—11·9	1·87	821	948	—13·3
G.G. ♂ 32-35	32-35	81·6	174	85·1	96·5	Jan. 8, '29	36·2	66	18·5	18·9	15·6	13	256							
25			*—1·9*	*110·0*				66					243							
													241							
Avg.								66					247	1,716	1,830	—6·2	1·97	871	948	—8·1
G.G. ♂ 32-35	32-35	81·6	174	85·1	96·5	Jan. 10, '29	35·8	60	18·5	18·3	15·0	13½	201							
30			*—1·9*	*110·0*				60					233							
Avg.								60					217	1,508	1,830	—17·6	1·97	765	948	—19·3

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height *and* $SH-\frac{H}{2}$	(SH) Sitting Height *and* Pelidisi	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Harris, Benedict, or Girl Scout.	Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.ct.	sq.m.	cal.	cal.	p.ct.
W.E. ♂ 20	20	61·9	163	78·7	81·3	Jan. 9, '29	36·0	54	17·0	16·1	14·4	11¼	159							
26			*+2·8*	*108·2*									192							
Avg.								54					176	1,223	1,468	—16·7	1·66	736	948	—22·3
P.K. ♂ 28	28	65·5	167	83·2	90·2	Jan. 10, '29	36·7	78	18·0	18·0	14·6	11¾	220							
28			*—0·3*	*105·0*				70					214							
Avg.								74					217	1,508	1,613	—6·5	1·74	867	948	—8·5
F.G. ♂ 35	35	59·9	172	81·9	86·4	Jan. 10, '29	36·1	60	19·0	18·0	14·6	12¾	190							
29			*+4·1*	*103·0*				60					221							
													192							
Avg.								60					191	1,327	1,514	—12·4	1·71	776	948	—18·1
C.K. ♂ 34	34	66·2	173	84·5	88·9	Jan. 11, '29	36·3	66	24·0	23·3	18·3	2½	235							
33			*—2·0*	*103·0*				66					219							
													228							
Avg.								66					227	—	—	—	—	—	—	—
C.H. ♂ 35	35	88·2	171	85·1	102·9	Jan. 10, '29	35·6	72	23·0	22·8	17·2	3½	222	—	—	—	—	—	—	—
31			*—0·4*	*113·0*																
B.J. ♀ 18	18	55·1	153	74·9	85·1	Jan. 1, '29	36·6	—	26·5	—	—	13½	159							
47			*—1·6*	*109·0*									159		1,201†	—8·0†				
Avg.													159	1,105	1,382	—20·0	1·51	732	912	—19·7
															1,250*	—16·6*				
W.W. ♂ 41	41	73·0	176	87·6	92·7	Jan. 4, '29	35·6	63	19·0	—	—	12	177							
51			*+0·4*	*102·0*									220							
Avg.								63					199	1,383	1,544	—10·4	1·88	735	924	—20·1
W.W. ♂ 41	41	73·0	150	87·6	92·7	Jan. 6, '29	36·1	62	17·5	—	—	12	210							
53			*+12·6*	*102·0*									210							
													215							
Avg.								62					212	1,473	1,544	—4·6	1·88	783	924	—15·2
E.P. ♂ 20	20	60·3	166	81·5	82·6	Jan. 4, '29	35·5	64	23·0	—	—	12½	178							
52			*+1·5*	*103·6*									184							
Avg.								64					181	1,258	1,466	—14·2	1·66	757	948	—20·1

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height *and* $SH-\frac{H}{2}$	(SH) Sitting Height *and* *Pelidisi*	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Harris, Benedict, or Girl Scout.	Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and Dubois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.ct.	sq.m.	cal.	cal.	p.ct.
H.Y. ♂ 54	27	68·9	162 *—0·9*	81·9 *108·0*	94·0	Jan. 7, '29	36·3	36	19·0	—	—	12½	196 212							
Avg.								36					284	1,417	1,517	—6·6	1·74	920	809	—14·6
L.W. ♂ 55	31	88·3	168·5 *—0·9*	85·1 *113·0*	102·9	Jan. 7, '29	36·0	62	19·0	—	—	12	278	1,932	1,887	+2·4	1·95	965	948	+1·8
L.W. ♂ 56	31	88·3	163 *+3·6*	85·1 *113·0*	102·9	Jan. 8, '29	36·0	64	19·5	18·3	16·1	12	279	1,938	1,887	+2·7	1·95	993	948	+4·7
L.W. ♂ 58	31	88·3	163 *+3·6*	85·1 *113·0*	102·9	Jan. 9, '29	35·4	60	17·1	16·7	15·0	12	269 272							
Avg.								60					271	1,883	1,887	—0·2	1·95	914	948	—3·5
L. ♀ 57	22	52·8	159 *—3·3*	76·2 *106·0*	78·7	Jan. 8, '29	35·6	68	20·0	20·0	16·4	12								
L. ♀ 61	22	52·8	159 *—3·3*	76·2 *106·0*	78·7	Jan. 10, '29	35·0	54	20·0	19·4	16·4	11¾	156 154							
Avg.								54					155	1,077	1,351	—20·3	1·53	704	888	—20·7
T.M. ♂ 59	28-30	76·0	145 *+1·8*	74·3 *123·0*	92·7	Jan. 9, '29	35·7	60	—	16·7	15·0	12½	245 245							
Avg.								60					245	1,702	1,641	+3·7	1·67	1,019	948	+7·5
P.K. ♂ 60	28	65·5	167 *—0·3*	83·2 *105·0*	90·2	Jan. 9, '29	36·1	72	—	—	—	12	209 218							
Avg.								72					214	1,487	1,613	—7·8	1·74	855	948	—9·8
K.B. ♂ 62	36	63·7	168 *—0·8*	83·2 *105·0*	87·6	Jan. 11, '29	36·6	102	17·7	16·1	11·4	12	279 271 271							
Avg.								102					274	1,904	1,539	+23·7	1·73	1,101	948	+16·1
F.G. ♂ 63	35	59·9	172 *—4·1*	81·9 *103·0*	86·4	Jan. 11, '29	35·6	76	—	—	—	11	202 194 196							
Avg.								76					197	1,369	1,514	—9·6	1·71	801	948	—15·5

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age.	Body Weight (without clothes)	(H) Height *and* $SH-\frac{H}{2}$	(SH) Sitting Height *and* *Pelidisi*	Chest Girth.	Date of Expt.	Mouth Temp.	Pulse.	Room Temp.	Outdoor Temp. Dry Bulb	Outdoor Temp. Wet Bulb	Hours without food.	O_2 per minute.	Heat produced per 24 hours. Total.	Harris, Benedict, or Girl Scout.	Deviation.	Body Surface (height-weight)	Heat (actual) per sq.m. per 24 hrs.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois.	Deviation of actual from Aub and DuBois prediction.
	yrs.	kg.	cm.	cm.	cm.		°C		°C	°C	°C		c.c.	cal.	cal.	p.ct.	sq.m.	cal.	cal.	p.ct.
C.K. ♂	34	66·2	173	84·5	88·9	Jan. 12, '29	36·2	66	21·5	16·1	14·7	13½	223							
64			—2·0	103·0									213							
								66					218	1,515	1,613	—6·1	1·79	846	948	—10·8
L.M. ♂	22	58·3	164	81·3	83·8	Jan. 5, '29	—	54	17·5	16·2	15·0	11½	176							
19			—0·7	103·0				54					215							
													184							
								54					192	1,334	1,540	—13·4	1·63	818	948	—13·7
C.H. ♂	35	88·2	171	85·1	102·9	Jan. 11, '29	35·6	54	17·0	19·4	16·1	12	212							
32			—0·4	113·0				54					212							
Avg.								54					212	1,473	1,898	—22·4	2·00	737	948	—22·3
C.H. ♂	35	88·2	171	85·1	102·9	—	36·1	60	19·5	16·1	14·6	12	198							
34			—0·4	113·0				60					199							
Avg.								60					199	1,383	1,898	—27·1	2·00	692	948	—27·0
E.J. ♂	15	49·9	159	79·4	76·8	Dec. 28, '28	36·1	68	20·0	—	—	12¼	266							
35			—0·1	100·0									240							
Avg.								68					240	1,668	1,447	+15·3	1·50	1,112	1,104	+0·7
E.J. ♂	15	49·9	159	79·4	76·8	Dec. 29, '28	36·0	54	—	—	—	13½	238	1,654	1,447	+14·3	1·50	1,103	1,104	—0·1
39			—0·1	100·0																
D.N. ♂	9	20·0	117	60·3	53·3	Dec. 29, '28	36·8	76	21·2	—	—	12½	187							
36			+1·8	98·0									168							
													170							
Avg.								76					175	1,216	866	+40·4				
D.N. ♂	9	20·0	117	60·3	53·3	Dec. 31, '28	37·2	84	31·5	—	—	12½	148							
44			+1·8	98·0									158							
Avg.								84					153	1,063	866	+22·7				
S. ♂	9	21·8	126	62·2	—	Dec. 29, '28	36·8	74	21·2	—	—	12	181							
37			—0·8	97·0									176							
													181							
Avg.								74					179	1,244	936	+32·9				

* Prediction based on average value of 1,250 calories found for groups (12 in group) of Girl Scouts from 12 to 18 years old. (Benedict.)
† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

Subject and Expt.	Age, yrs.	Body Weight (without clothes) kg.	(H) Height *and* $SH-\frac{H}{2}$ cm.	(SH) Sitting Height *and* *Pelidisi* cm.	Chest Girth. cm.	Date of Expt.	Mouth Temp. °C	Pulse.	Room Temp. °C	Outdoor Temp. Dry Bulb °C	Outdoor Temp. Wet Bulb °C	Hours without food.	O_2 per minute. c.c.	Heat produced per 24 hours. Total. cal.	Harris, Benedict, or Girl Scout. cal.	Deviation. p.ct.	Body Surface (height-weight) sq.m.	Heat (actual) per sq.m. per 24 hrs. cal.	Heat per sq.m. per 24 hrs., predicted by Aub and DuBois. cal.	Deviation of actual from Aub and DuBois prediction. p.ct.
F.C. ♂	15	37·4	151	76·2	69·9	Dec. 29, '28	36·2	60	17·5	22·8	17·8	12½	172							
38			+0·7	95·0									160							
													184							
Avg.								60					172	1,195	1,235	—3·2	1·27	941	1,104	—14·8
															1,053†	+22·7†				
E.H. ♀	19	48·3	140	71·1	82·6	Dec. 30, '28	36·8	72	15·5	—	—	12¼	186	1,292	1,287	+0·4	1·34	964	912	+5·7
40			+1·1	110·0											1,250*	+3·4*				
E.H. ♀	19	48·3	140	71·1	82·6	Jan. 1, '29	36·8	76	26·0	—	—	12¼	166							
45			+1·1	110·0									168		1,053†	+10·2†				
Avg.								76					167	1,160	1,287	—9·9	1·34	866	912	—5·0
															1,250*	—7·2*				
D.M. ♀	13	43·4	154	74·0	73·7	Dec. 31, '28	36·7	80	18·5	21·1	16·1	12¼	139							
41				102·3									132							
Avg.								80					136	945						
D.M. ♀	13	43·4	154	74·0	73·7	Jan. 2, '29	36·9	68	19·0	—	—	12½	167							
49				102·3									167							
Avg.								68					167	1,160						
D.M. ♀	13	43·4	154	74·0	73·7	Jan. 3, '29	36·7	70	25·5	—	—	12½	162							
50				102·3									160							
Avg.								70					161	1,119						
E.M. ♀	15	40·4	156	73·7	68·6	Dec. 31, '28	37·2	78	19·0	21·1	16·1	13	139							
42			—4·3	100·0				78					150		1,260†	—20·1†				
Avg.								78					145	1,007	962	+4·7	1·34	751	1,032	—27·2
															1,250*	—19·4*				
G.B. ♀	15	67·1	153	77·5	94·0	Dec. 31, '28	37·0	88	21·0	21·1	16·1	12	154							
43			+1·0	113·0									161							
Avg.								88					158	1,098	1,597†	—31·2†	1·65	665	1,032	—35·6
															1,250*	—12·2*				
G.B. ♀	15	67·1	154	77·5	94·0	Jan. 2, '29	37·3	70	27·0	—	—	14	155							
48			+0·5	113·0									155							
Avg.								70					155	1,077	1,597†	—32·6†	1·65	653	1,032	—36·7
															1,250*	—13·8*				
I.B. ♀	22	51·5	152	75·6	85·1	Jan. 1, '29	37·1	70	26·5	—	—	13	165	1,146	1,326	—13·6	1·47	780	888	—12·2
46			—0·4	106·0																

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† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

REMARKS ON TABLE.

1. Good subject. These two series were taken with a rest intervening. The subject had had four preliminary series done on previous days. He was quite used to the apparatus. Pulse 60 before and after experiment.

2. Good subject.

4. Very nervous subject. All females in this set had previously carried out a trial determination to insure familiarity with apparatus, and this is the first set recorded.

8. Subject good, though apprehensive so far as one could judge. Pulse steady in each series. Weather trying. Flies very bad.

5. Difficult to judge whether these subjects apprehensive or not. They answer only leading questions.

9. Good subject. Flies very bad. Uncomfortable at times, but practically no movement. Every care taken to reduce discomfort, which was acute for everyone.

10. Flies very bad. Subject excited. First series taken as soon as seemed ready. Nose clip failed and assistant held nose for last two series. His hand gave out at pump stroke 6 in last series.

15. Subject lay quiet but breathed somewhat irregularly. A nasal leak appeared in the second series, and in correcting it the subject became disturbed. A third attempt was not worth recording.

6. The weather was very trying. Flies were most persistent and spoiled the first series. An assistant kept them away in the last two experiments.

7. The nose of this subject was difficult to quickly occlude. After much difficulty one series of results was obtained, but all other attempts proved futile. Subsequently the nose was held by an assistant.

11. On account of previous difficulty with this subject she was taken one hour after food, having rested one hour. Compare with later results in post-absorptive state on January 2, 1929. "In the case of Irene (7, 11, and 13) there was a septic foot which we subsequently lanced. Despite this lesion, one of our best subjects. Stoicism amazing."

12. Very good subject. Done as in previous case afer breakfast. Compare with later results on 2-1-29.

14. Good subject, but somewhat distressed by the movement of other natives in vicinity. The second series undoubtedly the best conditions.

16. Flies very bad. Breathing curiously periodic in type, becoming better as experiment proceeds. The third series was begun after a rest and reassuring talk. The subject was a very good one.

17. A very good subject—lay like a corpse.

21. With these adults it was felt that it would be wise to release them at the earliest opportunity in order to favour their submitting at future examinations. In one or two cases they refused to submit again, and general dejection was feared if displeasure was provoked.

20. In series 2 the times for the third and fourth strokes of the gun are fused owing to necessity for asking other natives to leave the hut.

22. Subject almost totally deaf. Bone conduction very slight. Right tympanum perforate. Discovered this later, as well as the fact that under pressure he could force air quite easily through the right Eustachian tube. Physique otherwise splendid.

23. Great difficulty in stopping nostrils and ear leak (see above note). The results were obtained after about 35 minutes continuous experimentation.

24. Good subject. Hard-working native. Walked over from his hut where his family was (300 yards) and lay down in an empty hut under the bed clothes. He had rested in this manner 1¾ hours, and had had no food since previous day.

27. Subject walked over from family hut to experimental hut at 5 o'clock, and there went to bed again in his clothes and boots. He had thus rested for 1 hour 25 minutes and was in post-absorptive state.

25. Powerful subject. Very intelligent. Gave every assistance. Walked over from his own hut at 5 o'clock and rested in bed fully dressed until required in the experimental hut.

26. Good subject. This is the third series of estimations made upon him. The second will be reported separately. Disturbed during fifth gun of first series by other natives.

28. Not a good subject. Apprehensive. On two previous occasions overbreathed badly. At end of present series alveolar air=5·6%, indicating normal breathing. Impossible to prevent slight movement of hands and legs.

29. Very good subject. Amiable and obedient. Even in the previous preliminary work he showed no fear and was just as good a subject.

33. This subject, who was quite idle after breakfast, rested for 1½ hours prior to the estimation.

† Based on Table 21 in Publication 303A, Carnegie Institute, 2nd Edition.

31. First trial after one hour's rest at 11.30 a.m. He was very difficult to persuade, and although only one good series was obtained it was deemed worth including with the others. He was very intelligent, but also amiably stubborn. A big native actively employed on the farm.

32. These determinations were made in the hut in which this man and family lived. The wife left the room and an infant slept in the bed. There were no interruptions.

34. This subject is one of the heaviest yet examined. His weight is due to a fair degree of subcutaneous adiposity. At sixth gun, second series, a commotion set going by the infant in the same bed spoiled further work.

39. Not a good subject. Apprehensive and difficult to talk with.

36. This subject has been done (experiment No. 17) at a later date, but this is included to illustrate the type of results obtained after successive attempts.

44. High body temperature, but no apparent febrile condition.

37. Troublesome subject. Unable to keep him quiet prior to the estimation, so that he is not in a basal condition. Born of a 16-year-old mother. A peculiar ape-like type.

38. A good subject. Intelligent boy.

40. The second attempt of this subject. A better series is obtained later, January 1, 1929.

50. Compare No. 49. The original series would appear to be aberrant in the light of these later estimations.

40. This subject was an excellent one, but suffered from a feverish cold.

48. Compare with experiment No. 43. A heavy type of subject. "The heaviest type studied and very lethargic, although showing clinically no thyroid dystrophy."

46. Only one series utilized. Difficulty due to nasal leak. Subject otherwise quiet.

51. First attempt on Woxie. He appears to have overbreathed. Compare with later series.

53. Compare with first experiment, No. 51. A very tractable native.

52. Compare with experiment No. 20.

54. Curious temperament. Difficult to persuade him to co-operate and not clear whether he is apprehensive or not.

55. After many attempts on this big powerful native, easily the most powerful of the natives examined, this series was secured, and in the light of the subsequent estimations has some value. The main source of difficulty lay in the occlusion of the nostrils. Very good natured and highly intelligent.

56. Again illustrates the difficulty in securing good results. The nose was held by an assistant, but it was so cartilageous as to defeat even that device. The above are two series out of a total of six.

58. Again the same difficulty. Although the subject seemed perfect, only two (2) out of a long series were reproducible.

57. These are the figures obtained from the only married "lubra" who would submit. She was very stoical and had been brought up at the mission from childhood. The other lubras refused all co-operation.

59. Compare with experiment No. 22.

60. This subject walked over at 5 o'clock from his own hut to the experimental hut and then went to bed again. He was examined at 7.30, so that he had rested for 2½ hours.

63. Very tractable subject.

64. Compare with experiment No. 33.

19. Very good subject. He was disturbed during the second series by other natives coming and looking on. These were sent away and the final series then proved satisfactory. Unfortunately the subject left the camp next day.